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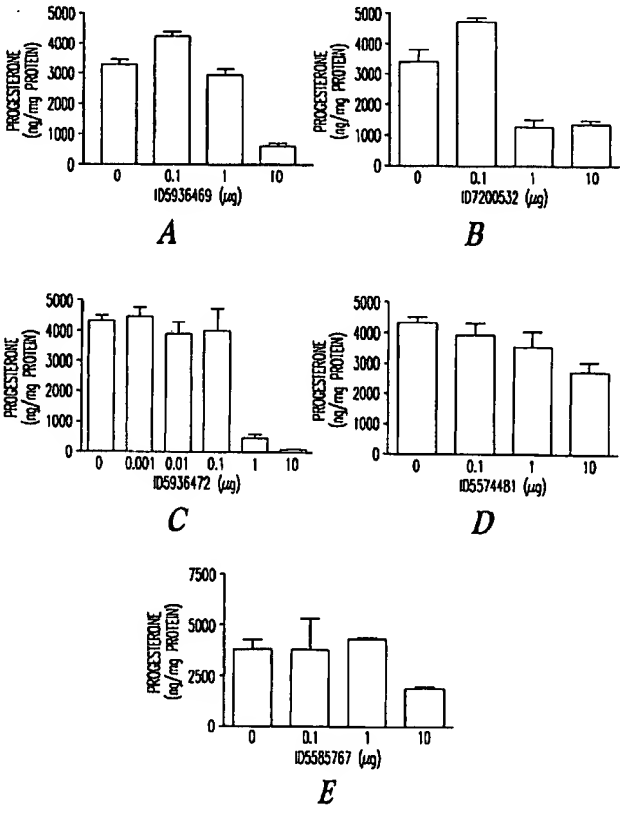
(54) Title: STRUCTURE BASED DRUG DESIGN OF STEROIDOGENESIS INHIBITORS




(57) Abstract: Steroidogenesis begins with the transfer of free cholesterol from intracellular stores into mitochondria. The peripheral-type benzodiazepine receptor (PBR) and steroidogenic acute regulatory protein (StAR) function in this process, functioning together to shuttle cholesterol from outside the mitochondria to the inner mitochondrial membrane. The present invention provides methods of using structure-based drug design to identify peptide and substituted steroid compounds that can inhibit the activity of PBR and StAR via interaction with their cholesterol-binding domains. These approaches facilitated the identification of a number of inhibitory ligands that decrease steroid biosynthesis.

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# STRUCTURE BASED DRUG DESIGN OF STEROIDOGENESIS INHIBITORS

## Cross Reference to Related Applications

This application claims the benefit of the filing date of U.S. application Serial No. 60/778,032, filed on Mar 1, 2006, and U.S. application Serial No. 60/745,693, filed on Apr 26, 2006, the disclosures of which are incorporated by reference herein.

## Background

Steroids are a large family of structurally similar lipid substances, characterized by a basic skeleton consisting of four interconnected carbon rings. Naturally-occurring steroids function as hormonal messengers in the body, regulating and maintaining reproductive systems, metabolism, immune function, blood volume, excretion of electrolytes, and muscle and bone mass. Elevated steroid levels have been linked to many diseases, including cancers and neurological disorders.

Steroid biosynthesis begins with the transfer of free cholesterol from intracellular stores into mitochondria. The first enzymatic step in steroidogenesis, the conversion of cholesterol to pregnenolone, is catalyzed by cytochrome P450 side chain cleavage (P450scc) in the inner mitochondrial membrane. Pregnenolone then leaves the mitochondrion to undergo enzymatic transformation in the endoplasmic reticulum where it can be metabolized to other steroid products. The transport of cholesterol from the outer to the inner mitochondrial membrane is thus the rate-determining step in steroid and bile acid biosynthesis.

The peripheral-type benzodiazepine receptor (PBR) and the steroidogenic acute regulatory protein (StAR) play significant roles in intramitochondrial cholesterol transport. Although the exact mechanism by which these proteins function in cholesterol transport has not yet been fully elucidated, much evidence suggests that these proteins function together to shuttle cholesterol from outside the mitochondria to the inner mitochondrial membrane for steroid synthesis.

PBR is particularly abundant in steroid-producing tissues, where it localizes to the outer mitochondrial membrane and the outer/inner membrane contact sites. PBR binds cholesterol with high affinity. Additionally, PBR binds ligands which cause its activation. PBR is thought to be a cholesterol transporter/exchanger, bringing cholesterol to the steroid synthesis enzymes on the matrix side of the inner mitochondrial membrane.

Likewise, the StAR protein regulates the limiting step in steroid hormone production in response to hormonal stimuli by transferring cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, likely as a result of its direct interaction with the outer surface of the mitochondria.

Recent studies have suggested that these two proteins interact under physiological conditions and that PBR in the outer mitochondrial membrane mediates StAR-induced cholesterol import into mitochondria. This functional interaction, wherein PBR serves as a gatekeeper in protein and cholesterol import into mitochondria and StAR serves as a hormone-induced activator, is likely required for the subsequent formation of a variety of steroids.

What is needed are methods and compositions that decrease steroid levels, specifically by regulating steroidogenesis.

## Summary of the Invention

One embodiment of the invention provides methods to inhibit steroidogenesis in a cell (e.g., in vitro or in vivo) or subject with a compound that binds to a START or CRAC domain in an amount effective to inhibit steroidogenesis in said cell (e.g., cells in culture, such as mammalian, including stem cells) or subject.

One embodiment provides a method to inhibit activity of a START or CRAC domain, comprising contacting a START or CRAC domain with a compound that binds to the START or CRAC domain and thereby inhibits the activity of said START or CRAC domain.

Another embodiment provides a method to inhibit interaction between cholesterol and a CRAC or START domain comprising administering a compound that binds to a CRAC or START domain in an amount effective to inhibit the interaction between said cholesterol and said CRAC or START domain.

Another embodiment provides a method to treat a condition that is characterized by overproduction of a steroid in a subject in need thereof, comprising administering a compound that binds to a START or CRAC domain in an amount effective to treat said condition.

In one embodiment, the steroidogenesis produces a steroid selected from the group consisting of pregnenolone and progesterone. In another embodiment, the steroidogenesis produces a steroid selected from the group consisting of pregnenolone and progesterone metabolites. In one embodiment, the steroidogenesis produces a steroid selected from the group consisting of dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone sulfate, aldosterone, testosterone, dihydrotestosterone, 3α-hydroxy-5α-pregnane-20-one (allopregnanalone), 5α-pregnan-3 α-ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, and 11-deoxycorticosterone.
In one embodiment, the START domain is present in a protein selected from the group consisting of STARD3/MLN64, STARD1/StAR, STARD4, STARD5, STARD6, STARD11/CERT, STARD10, STARD7, STARD2/PCTP, STARD12/DLC-1, STARD13/DLC-2, STARD8, STARD15/CACH, STARD14/BFIT, and STARD9. In one embodiment, the START domain comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

In another embodiment, the CRAC domain is present in peripheral-type benzodiazepine receptor. In one embodiment, the CRAC domain comprises a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

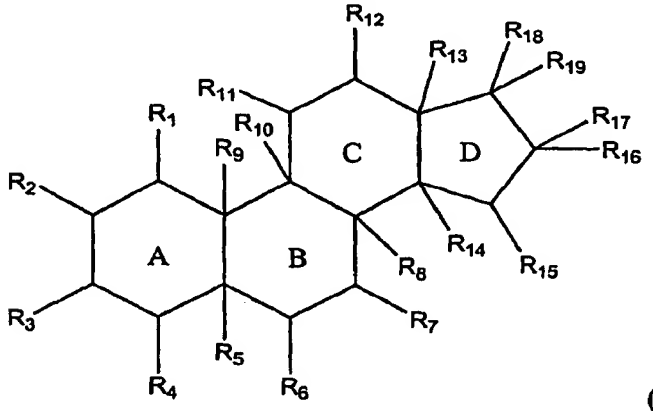
In one embodiment, the compound is a polypeptide selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19.

In one embodiment, the steroid is selected from the group consisting of pregnenolone and progesterone. In another embodiment, the steroid is selected from the group consisting of pregnenolone and progesterone metabolites. In one embodiment, the steroid is selected from the group consisting of pregnenolone,

progesterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone sulfate, aldosterone, testosterone, dihydrotestosterone, 3α-hydroxy-5α-pregnane-20-one (allopregnanalone), 5α-pregnan-3 α -ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, and 11-deoxycorticosterone.

In one embodiment, the condition is selected from the group consisting of glioma, neurodegenerative disorder, brain injury, brain inflammation, Alzheimer's disease, ischemia-reperfusion injury, epilepsy, affective disorder, fatigue during pregnancy, premenstrual syndrome, postpartum depression, catamenial epilepsy, alcoholism, sleep disorder, memory disorder, premenstrual dysphoric disorder, mood disorder, depressive disorder, anxiety disorder, eating disorder, dementia, stress disorder, aggressiveness, convulsions, pain, neuronal degeneration, neurite outgrowth disorder, synaptogenesis disorder, hyperpituitarism, multiple sclerosis, Parkinson's disease, Huntington's disease, hepatic encephalopathy, peripheral nerve degeneration, adrenal hyperplasia, gonadal hyperplasia, and colon carcinoma.

In another embodiment, the compound has the structure of Formula (I):




(I)

wherein:

fused rings A, B, C, and D are independently saturated or fully or partially unsaturated; and

$R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ each independently comprises hydrogen, hydroxyl, oxo, halogen, cyano, nitro, carboxy, substituted or unsubstituted amino, substituted or unsubstituted ($C_1$-

$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) hydroxyalkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyloxy-($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarboxy-($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{20}$) alkyl, or -$SO_2A$, wherein A comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl;

or $R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ together with the atoms they are attached can form a substituted or unsubstituted carbocyclic or a substituted or unsubstituted heterocyclic ring, wherein the substituted carbocyclic or the substituted heterocyclic ring may be substituted with one or more substituted or unsubstituted carbocyclic rings or one or more substituted or unsubstituted heterocyclic rings;

or $R_{16}$ and $R_{17}$ may be taken together as $=C(R_aR_b)$, wherein $R_a$ and $R_b$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl;

or $R_{18}$ and $R_{19}$ may be taken together as $=N\text{-}N(R_cR_d)$, wherein $R_c$ and $R_d$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, heterocyclylcarbonyl, or ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl; and

$R_5$, $R_8$, $R_9$, $R_{10}$, $R_{13}$, and $R_{14}$ is each independently: deleted when one of fused rings A, B, C, or D is unsaturated so as to complete the valency of the carbon atom at that site, or

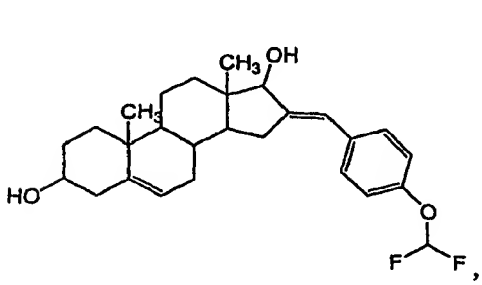
each independently comprises hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) hydroxyalkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyloxy-($C_1$-$C_{18}$) alkyl;

or a pharmaceutically acceptable salt thereof.

For example, in one embodiment, $R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ each independently comprises hydrogen, hydroxyl, oxo, methyl, ethylenedioxy, cyano, $-CH_2OH$, $-OCOCH_3$, $-COCH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2COOH$, $-COCH_2OCOCH_3$, $-COCH_2OH$, -COOH, $-CO(CH_3)COCH_2CH_2CH_2CH(CH_3)_2$, or $-OCOCH_3$; or $R_{16}$ and $R_{19}$ together with

the atom or atoms to which they are attached can form a tetrahydrofuran ring; or $R_{16}$ and $R_{17}$ may be taken together as $=C(R_aR_b)$, wherein $R_a$ and $R_b$ each independently comprises hydrogen, p-nitrophenyl, p-methoxyphenyl, or 2-(5-methyl furfuryl); or $R_{18}$ and $R_{19}$ may be taken together as $=N\text{-}N(R_cR_d)$, wherein $R_c$ and $R_d$ each independently comprises hydrogen or –C(O)-3-pyridene; and $R_5$, $R_8$, $R_9$, $R_{10}$, $R_{13}$, and $R_{14}$ is each independently: deleted when one of fused rings A, B, C, or D is unsaturated so as to complete the valency of the carbon atom at that site, or each independently comprises hydrogen, hydroxyl, or methyl.

In another embodiment, the compound is

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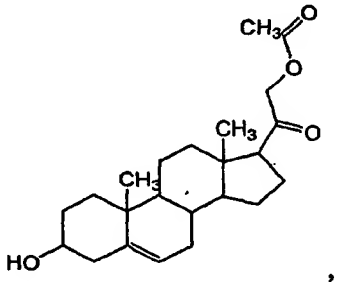


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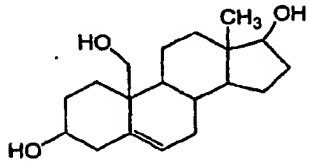
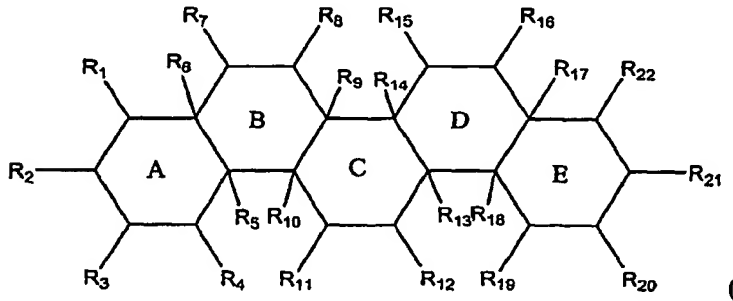



In another embodiment, the compound has the structure of Formula (II):



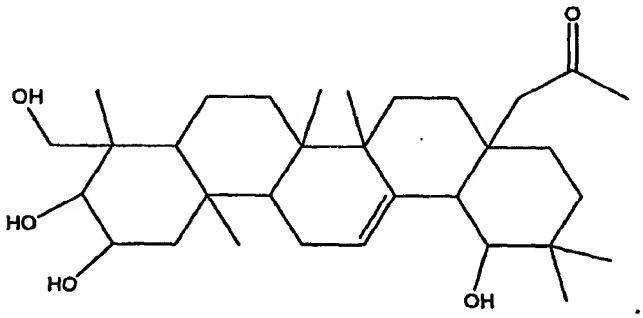

(II)

wherein: fused rings A, B, C, D, and E are independently saturated or fully or partially unsaturated; and $R_1$ through $R_4$, $R_6$, $R_7$, $R_8$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{19}$, $R_{20}$, $R_{21}$, and $R_{22}$ each independently comprises hydrogen, hydroxyl, oxo, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, or substituted or unsubstituted ($C_1$-$C_{18}$)

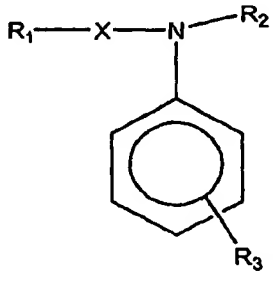
hydroxyalkyl; or $R_5$, $R_6$, $R_9$, $R_{10}$, $R_{13}$, $R_{14}$, $R_{17}$, and $R_{18}$ is each independently: deleted when one of fused rings A, B, C, D, or E is unsaturated so as to complete the valency of the carbon atom at that site, or each independently comprises hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl; or a pharmaceutically acceptable salt thereof.

For example, in one embodiment, $R_1$ through $R_4$, $R_6$, $R_7$, $R_8$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{19}$, $R_{20}$, $R_{21}$, and $R_{22}$ each independently comprises hydrogen, hydroxyl, methyl, hydroxymethyl, or gem-dimethyl, gem-methyl, hydroxymethyl; or $R_5$, $R_6$, $R_9$, $R_{10}$, $R_{13}$, $R_{14}$, $R_{17}$, and $R_{18}$ is each independently: deleted when one of fused rings A, B, C, D, or E is unsaturated so as to complete the valency of the carbon atom at that site, or each independently comprises hydrogen, hydroxyl, or $-CH_2COCH_3$.

In one embodiment, the compound is




In another embodiment, the compound has the structure of Formula (III):




(III)

wherein X is a carbonyl or a direct bond;$R_1$ comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl; $R_2$ comprises of hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl($C_1$-$C_{18}$) alkyl, substituted

or unsubstituted cyclo($C_3$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl; $R_3$ comprises mono-, di-, tri, tetra, or penta-substituted independently with halogen, hydrogen, hydroxyl, ($C_1$-$C_{18}$)alkoxy, ($C_1$-$C_{18}$)alkylthio, -N($R_a$)($R_b$), nitro, cyano, carboxy, ($C_1$-$C_{18}$)alkyloxycarbonyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarbonyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl($C_1$-$C_{18}$) alkyl, substituted or unsubstituted cyclo($C_3$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or -$SO_2$NHA, wherein A comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarbonyl, and further wherein $R_a$ and $R_b$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted cyclo($C_3$-$C_{20}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl; or a pharmaceutically acceptable salt thereof.

For example, in one embodiment, X is a carbonyl; $R_1$ comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl; $R_2$ comprises hydrogen; and$R_3$ comprises halogen or carboxy. In another embodiment, X is a direct bond; $R_1$ comprises substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl; $R_2$ comprises hydrogen; and $R_3$ comprises halogen or carboxy.

One embodiment provides a method to screen for a compound which blocks interaction between cholesterol and a START domain comprising contacting the compound with cholesterol and a START domain; and determining whether a decrease in the level of interaction between the cholesterol and the START domain occurs in response to the compound, wherein a decrease in interaction indicates that the compound blocks the interaction between cholesterol and said START domain.

One embodiment provides a computer-assisted method for identifying compounds that have binding affinity to a CRAC or START domain, comprising the steps of identifying the probable lipid binding pocket sites in the CRAC or START domain, optimizing the probable lipid binding pocket sites using molecular mechanics calculations, screening a database of chemical compounds

for molecular docking activity with the CRAC or START domain, ranking the compounds in the database based on the results of a consensus scoring function, and selecting the compounds having high scores. In one embodiment, the START domain is a consensus sequence generated by combining the human STARD3 and mouse STARD4 structures.

In one embodiment, the identification of the probable lipid binding pocket sites is performed by the biopolymer module and FlexX programs of Sybyl 7.0 software. In another embodiment, the optimization of the probable lipid binding pocket sites is performed by the Simulated Annealing method by the Affinity module of InsightII software. In one embodiment, the screening is performed by the FlexX program of Sybyl 7.0 software.

In one embodiment, the consensus scoring function is a combination of the molecular mechanics energy, surface area, and a statistical parameter derived from known ligand-protein x-ray crystal structure complexes. In another embodiment, compounds having high consensus scores are further subjected to energy minimization using CFF91 force field molecular mechanics calculations to better fit said compounds having high scores into said lipid binding pocket sites.

In one embodiment, the method further comprises treating cells that conduct steroidogenesis with said compounds having high scores and testing the cells for inhibition of steroid synthesis.

Another embodiment provides a compound to prepare a medicament to inhibit steroidogenesis, wherein the compound binds to a START or CRAC domain.

## Brief Description of the Figures

Figure 1. Molecular surface area of the homology-modeled human CRAC peptide, mapped with residues A147 to N158. The cholesterol binding site is a deep cleft formed between Y152 and R156. The two hydrogen bond donors of Y152 and R156 were used for the pharmacophore search.

Figure 2. Identified pharmacophore atoms and their chemical properties (H-Bond donor site, Acceptor site and Hydrophobic nature). The pharmacophoric templates were utilized to conduct a 2D UNITY search of

various databases. To define the pharmacophores, the acceptor and donor sites of the Flex pharmacophores were defined without distance constraint. By using the receptor site module, exclusion spheres were defined up to 5Å from the pharmacophore site to get appropriately docked structures.

Figure 3. Binding mode of Compound 1 with CRAC peptide. The hydrogen bonding interactions are represented as dotted lines; the ligand atoms are shown as ball and stick models. The dotted lines indicate the intermolecular hydrogen bond interactions with Y152 and R156.

Figure 4. Inhibition of steroid synthesis by CRAC ligands in MA-10 Leydig cells. Synthesis of endogenous progesterone was decreased by all the compounds tested, thus confirming the docking simulation. The ID numbers beneath the graphs correspond to the ligand structures shown in Figure 9. A) ID5936469 = compound 2; B) ID7200532 = compound 4; C) ID5936472 = compound 5; D) ID 5574481 = compound 7; E) ID 5585767 = compound 8.

Figure 5. Identified pharmacophores on the designed peptide structure showing distance constraints. To identify peptidomimetic structures, one of the high binding affinity peptide structures underwent pharmacophore searches with distance constraints.

Figure 6. Sequence alignment of MLN-64 amino acids 76-223 (SEQ ID NO:1; GenomeId 6225682), StARD4 amino acids 128-275 (SEQ ID NO:2; GI 170459), HstAR amino acids 128-275 (SEQ ID NO:3; GI 1351124) and MstAR amino acids 127-274 (SEQ ID NO:4; GI 1236243). Residues occupying the lipid-binding region are marked with different shade backgrounds and text color (for MLN-64, gray boxes with black text; for StARD4, black boxes with white text; for HstAR and MstAR, gray boxes with black text). Residues that are involved in hydrogen bonding and hydrophobic interactions in all StAR domains are outlined by black lines.

Figure 7. Structures of StAR domain. A) Optimized structure of H/M StAR domain from cholesterol-StAR minimized complex by SA Docking. Small spheres indicate the binding pockets. B) FlexX docked steroid structure at cholesterol binding site. C) Intermolecular interactions of identified high affinity structure at cholesterol binding site. Hydrogen-bonding interactions are shown by dotted lines.

Figure 8. Residues at the cholesterol binding site used for the pharmacophore search are represented by stick models, where hydrophobic sites are represented by horizontal lines and hydrogen bond acceptor sites are represented by vertical lines.

Figure 9. Identified high affinity binding structures from molecular docking and minimization methods.

Figure 10. Biological activity studies of compounds 1-8. Figures show progesterone formation in MA-10 cells. Cells were treated for 3 hr with the indicated doses of compounds for 3 hours at 37°C, then followed by additional 2 hours incubation with 1 mM dibutyryl cAMP per well. The media was collected for progesterone measurement by RIA and cells were collected to determine protein levels. Data shown are means ± SEM from an experiment performed in triplicates.

Figure 11. Inhibition of steroid synthesis by CRAC ligands in MA-10 Leydig cells. Figures show progesterone formation in MA-10 cells.

## Detailed Description of the Invention

### I. Steroids

Steroids are a large family of structurally similar lipid substances, characterized by a basic skeleton consisting of four interconnected carbon rings. Naturally-occurring steroids function as hormonal messengers in the body, regulating and maintaining reproductive systems, metabolism, immune function, blood volume, excretion of electrolytes, and muscle and bone mass. Elevated steroid levels have been linked to many diseases, including cancers and neurological disorders. Steroids can function by both genomic and non-genomic effects. The genomic effects of steroids are mediated through protein members of the superfamily of steroid hormone receptors, a group of intracellular transcription factors (Beato, 1989).

In the central nervous system (CNS), steroids modulate membrane-bound neurotransmitter receptors (Majewska, 1987; Lambert et al., 1995), including the GABA(A) receptor complex, the NMDA class of glutamate receptors, and sigma receptors (Baulieu, 1997; Mellon and Griffin, 2002). Because of their lipophilic structure, steroids can easily diffuse across the blood-brain barrier if administered peripherally, thereby bypassing the issues of drug delivery from the

circulation across the blood-brain barrier and into the brain. Furthermore, the amounts of steroid sufficient to induce changes in neural activity are extremely low, typically in the nanomolar range.

In the reproductive system, the sex steroid hormones estrogen, progesterone and androgen play pivotal roles in sex differentiation and development, and in reproductive functions and sexual behavior. The estrogen receptors, progesterone receptors and androgen receptor are modulated by the sex steroid hormones.

Steroids also exert non-genomic effects, particularly in the brain (Selye, 1941, 1942). These effects were initially shown to involve anesthetic metabolites of progesterone, but have since been expanded to include a large number of steroid compounds. These non-genomic activities are characterized by extremely rapid effects, lasting from milliseconds to minutes, and do not require interaction with steroid hormone receptors (McEwen, 1994).

Steroidogenesis

Steroids are formed by several successive enzymatic transformations of cholesterol. The first step takes place in the inner mitochondrial membrane and is the conversion of cholesterol to pregnenolone, catalyzed by the cholesterol side chain cleavage cytochrome P450 (P450scc; CYP11A1). Pregnenolone then leaves the mitochondrion and moves towards the endoplasmic reticulum, where it undergoes further enzymatic transformation, giving rise to the final steroid products (Lacapere and Papadopoulos, 2003).

Steroidogenesis is regulated by trophic hormones such as adrenocorticotropic hormone (ACTH) in adrenocortical cells and luteinizing hormone (LH) in testicular Leydig and ovarian cells. These peptide hormones bind to their specific plasma membrane G protein-coupled receptors, activating the stimulatory G protein. A stimulation of adenylyl cyclase follows, which results in an increase in cAMP. The rise of cAMP levels in the cells induces three responses: 1) lipid synthesis; 2) protein synthesis; and 3) phosphorylation of specific proteins (Simpson et al., 1983; Hall, 1985; Kimura, 1986; Waterman and Simpson, 1989; Orme-Johnson, 1990; Jefcoate, 2002). These intracellular changes trigger the transfer of cholesterol from storage or synthesis sites to the mitochondria. The transport of cholesterol within the mitochondria, from the

outer to inner mitochondrial membrane, forms the rate-determining step of steroidogenesis.

Two proteins play a role in intramitochondrial cholesterol transport: the peripheral-type benzodiazepine receptor (PBR) and the steroidogenic acute regulatory protein (StAR). In situ and in vitro studies indicated that in steroidogenic cells, StAR-induced cholesterol import into mitochondria was mediated by PBR in the outer mitochondrial membrane (Hauet et al., 2005), indicating that there is a functional interaction between StAR and PBR for cholesterol delivery into mitochondria and subsequent steroid formation. The association of StAR and PBR in a cell system was recently demonstrated (West et al., 2001). It has been proposed that PBR serves as a gatekeeper in protein and cholesterol import into mitochondria and that StAR serves the role of the hormone-induced activator, both proteins working in concert to bring cholesterol from intracellular stores into mitochondria (Hauet et al., 2005). While not being limited by theory, it is thought that the cholesterol is transferred from StAR to PBR, then subsequently transported across the outer to the inner mitochondrial membrane upon PBR activation by ligands (McEnemy et al., 1992; Lacapere and Papadopoulos, 2003).

It is the object of the instant invention to provide methods and compositions that alter steroid levels, e.g., decrease steroid levels. Specifically, the instant invention provides methods and compositions that alter steroid levels by disrupting steroidogenesis. The methods and compositions described herein provide ligands that bind to functional domains in the PBR and StAR, thus disrupting their function in steroidogenesis. These ligands include peptides, synthetic peptides, and peptidomimetic small molecules.

Steroids whose levels are contemplated, in one embodiment, to be reduced or eliminated by the methods and compounds described herein include, but are not limited to: neurosteroids, steroid hormone intermediates, dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone, pregnenolone sulfate, all $C_{18}$, $C_{19}$ and $C_{21}$ steroids, progesterone, metabolites of progesterone, androgens, estrogens, glucocorticoids, mineralocorticoids, progestagens, aldosterone, testosterone, dihydrotestosterone, 3$\alpha$-hydroxy-5$\alpha$-pregnane-20-one (allopregnanalone), 5$\alpha$-pregnan-3 $\alpha$-ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-

progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, 11-deoxycorticosterone, and other steroid intermediates in the pathway of pregnenolone formation.

By virtue of reducing or eliminating the levels of steroids, the methods and compositions described herein are contemplated to reduce the effects caused by cholesterol-derived steroids. These effects include genomic and non-genomic, receptor-mediated, and non-receptor-mediated effects of steroids.

Steroid receptor-mediated activity that is contemplated to be reduced or eliminated by the methods and compositions described herein includes, but is not limited to: activity of receptors such as GABA(A) receptors, N-methyl-d-aspartate (NMDA) receptors, nicotinic receptors, muscarinic receptors, glutamate receptors, serotonin (5-HT(3)) receptors, kainate receptors, glycine receptors, sigma receptors, glycine-activated chloride channels, nicotinic acetylcholine receptors, voltage-activated calcium channels, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, estrogen receptors, progesterone receptors, and androgen receptors.

Conditions treated by the methods and compositions of the invention include, but are not limited to, glioma, neurodegenerative disorders, brain injury, brain inflammation, Alzheimer's disease, ischemia-reperfusion injury, epilepsy, affective disorders, fatigue during pregnancy, premenstrual syndrome, postpartum depression, catamenial epilepsy, alcoholism, sleep disorders, memory disorders, premenstrual dysphoric disorder, mood disorders, depressive disorders, anxiety disorders, eating disorders, dementia, stress disorders, aggressiveness, convulsions, pain, neuronal degeneration, neurite outgrowth disorders, synaptogenesis disorders, hyperpituitarism, multiple sclerosis, Parkinson's disease, Huntington's disease, hepatic encephalopathy, peripheral nerve degeneration and regeneration, adrenal hyperplasia, gonadal hyperplasia, colon cancer, breast cancer and prostate cancer.

**II. Peripheral-type Benzodiazepine Receptor**

The peripheral-type benzodiazepine receptor/recognition site was identified initially in 1977 when investigators were searching for binding sites for the benzodiazepine diazepam in peripheral tissues. The early characterization of these diazepam binding sites outside the brain led to their

assignment as "peripheral-type" benzodiazepine receptor, or PBR, to distinguish them from the central benzodiazepine receptor (CBR), which is part of the GABA(A) receptor complex (Gavish et al., 1999; Lacapere and Papadopoulos, 2003).

PBR is a widely distributed transmembrane protein located mainly in the outer mitochondrial membrane. PBR binds with high affinity to drug ligands and cholesterol (Lacapere et al., 2001). PBR ligands stimulate cholesterol transport from outer to inner mitochondrial membrane and subsequent cholesterol-supported steroidogenesis (Python et al., 1993; Tsankova et al., 1995). Many functions are associated directly or indirectly with the PBR, including the regulation of cholesterol transport and synthesis of steroid hormones, porphyrin transport and heme synthesis, apoptosis, cell proliferation, anion transport, regulation of mitochondrial functions, and immunomodulation. Based on these functions, there are many clinical applications of PBR modulation, including oncologic, endocrine, neuropsychiatric, and neurodegenerative disease applications. A review of the various functions of PBR is presented below.

Expression and cellular localization. At the subcellular level, the localization of the PBR is mainly in the outer mitochondrial membrane (OMM), and especially at outer/inner mitochondrial membrane contact sites (Lacapere and Papadopoulos, 2003). The association of PBR with the mitochondrial permeability transition pore (MPTP) has been suggested, although whether it is a true component of the PBR complex or simply coincidentally associated with the MPTP is not yet known. Although PBR ligands have been shown to regulate MPTP (Kinnally et al., 1993), it remains to be determined if PBR has an active role or passive association with the MPTP.

The subcellular localization of the PBR is not always in the OMM. Venturini *et al.* demonstrated for the first time the presence of the PBR in the nucleolus of normal and cancerous liver human tissues (Venturini et al., 1998), and subsequently it has also been observed in the perinuclear area and nucleus of cancer cells (Hardwick et al., 1999), where a correlation of nuclear localization of the receptor with the rate of cell proliferation was suggested. Moreover, PBR also has been found to be distributed in the plasma membrane and other organelle membranes of various cell types (Olson et al., 1988; Oke et al., 1992).

Although different subcellular localization of PBR may reflect different functions of the protein in normal and pathologic conditions, the significance of these observations has not yet been firmly established.

Receptor complex structure: PBR and associated proteins. In the outer mitochondrial membrance and in the outer/inner mitochondrial membrane contact sites, the PBR is a component of a multimeric 140- to 200-kDa complex (Lacapere and Papadopoulos, 2003). The following proteins have been identified in this PBR complex: the 18-kDa pk18 IBP (binding site for isoquinoline carboxamides) (Antkiewicz-Michaluk et al., 1988; Lacapere et al., 2001), the 32-kDa voltage-dependent anion channel (VDAC), for conferring benzodiazepine binding (McEnery et al., 1992; Garnier et al., 1994), and the 30-kDa adenine nucleotide translocase (ANT; McEnery et al.,1992), of as yet unidentified function. Furthermore, two other proteins have been found to associate with PBR: PRAX-1 (PBR-associated protein 1; Galiegue et al., 1999) and PAP-7 (PBR- and protein kinase A [RIα-associated protein 7) (Li et al., 2001).

Although VDAC is required for binding of diazepam binding inhibitor (DBI; endozepine) and benzodiazepines (BZDs) to the PBR, which is the only protein that contains binding sites for isoquinolines (Antkiewicz-Michaluk et al., 1988; Lacapere et al., 2001), subsequent studies identified an 18-kDa protein, the PBR monomer, as the minimal functional unit binding drug ligands and cholesterol with nanomolar affinities (Lacapere et al., 2001). Interaction between the various proteins of the complex may drive its drug-binding properties and related function(s) (Golani et al., 2001). For example, homopolymerization of PBR monomers may occur through crosslinkages between tyrosine amino acid residues (Tyr-Tyr) to alter pharmacologic or physiologic properties (Delavoie et al., 2003), while the heterotypic association with other proteins such as VDAC may further limit some of these properties as well.

Endogenous and exogenous ligands. PBR ligands do not have a typical shared structure. Among those described are BZD derivatives, isoquinoline carboxamide derivatives, 2-aryl-3-indoleacetamide derivatives, pyrrolobenzoxazepine derivatives, 2-phenyl-imidazo[l,2-a]pyridine derivatives, phenoxyphenyl-acetamide derivatives, pyridazinoindole derivatives, and 8-

oxodihydropurine derivatives (Pedigo et al., 1981; Le Fur et al., 1983; Romeo et al., 1992; Campiani et al., 1996; Serra et al., 1999; Culty et al., 2001; Trapani et al., 2005; Kita et al., 2004).

In addition to the high-affinity PBR drug ligands described above, dicarboxylic porphyrins bind to PBR, and although they show lower affinity, they have been proposed to serve as endogenous ligands (Snyder et al., 1987). The polypeptide diazepam binding inhibitor (DBI) was identified also as a candidate endogenous PBR ligand (Lacapere and Papadopoulos, 2003; Costa and Guidotti, 1991). Naturally occurring DBI processing products were also shown to be PBR ligands. More recently, cholesterol was shown to be a high-affinity endogenous ligand for PBR (Lacapere and Papadopoulos, 2003).

Radioligand binding and expression studies demonstrated that the rodent 18-kDa PBR protein contains the determinants for both isoquinoline carboxamide and BZD binding (the extent of which depends on VDAC). However, human and bovine proteins exhibit similar isoquinoline carboxamide binding characteristics to the rodent protein but 50- to 100-fold lower affinity for BZDs (Gavish et al., (1999); Farges et al., 1993; Parola and Laird, 1991). These species differences most likely can be attributed to differences in the primary amino acid sequence (Farges et al., 1993); the evolutionary significance of these differences is not well understood.

Fundamental functions attributed to the PBR

A body of evidence supports three main potential structure-function relationships for the PBR: cholesterol binding followed by cholesterol transport in steroid and bile salts biosynthesis; protein import useful in membrane biogenesis; and porphyrin binding and transport involved in heme biosynthesis. All other identified functions of PBR may be due, directly or indirectly, to its cholesterol and/or porphyrin binding ability and/or to its role in cholesterol transport, porphyrin transport and/or protein import.

Steroid biosynthesis. Synthesis of steroids is an extensively studied PBR function. Diazepam binding inhibitor (DBI) and its proteolytic products interact with the PBR to stimulate the synthesis of steroids in the mitochondria. Ligands of the PBR have been shown to stimulate steroidogenesis and neurosteroidogenesis *in vitro* and *in vivo* and have been shown to act by facilitating mitochondrial cholesterol delivery (cholesterol translocation from the

outer to the inner mitochondrial membrane), which results in increased cholesterol metabolism to pregnenolone by cytochrome P450 C27 side-chain cleavage enzyme (P450scc; CYP11A1), the rate-determining step in the synthesis of steroids (Lacapere and Papadopoulos, 2003; Papadopoulos et al., 2006; Costa et al., 1994).

Experimental evidence suggests that the cholesterol recognition amino acid consensus (CRAC) domain in the cytosolic C-terminal domain of the PBR may be the binding site responsible for the mitochondrial uptake and translocation of cholesterol (Li et al., 2001; Jamin et al., 2005). These studies also demonstrated that PBR is a high-affinity cholesterol-binding protein. The role of the PBR in steroidogenesis also has been demonstrated in knockout and antisense experiments in which the expression of PBR was down-regulated (Lacapere and Papadopoulos, 2003).

Deletion mutations on recombinant PBR have shown that various truncated forms expressed reduced ability to bind ligand PK 11195, whereas cholesterol uptake was maintained. However, deletion of the C-terminus of PBR (Δ153-169) drastically reduced cholesterol uptake (70%), although it retained ability to bind PK 11195 ligand (Li and Papadopoulos, 1998), indicating that PBR had distinct cholesterol and ligand-binding sites. Site-directed mutagenesis in this 153-169 region enabled the characterization of amino acids involved in cholesterol binding. A CRAC sequence has been determined: ATVLNYYVWRDNS (SEQ ID NO:5). This amino acid consensus pattern has been observed in several other proteins known to interact with cholesterol (Li and Papadopoulos, 1998). The role of PBR in the synthesis of steroids is thus firmly established.

CRAC has the ability to bind cholesterol with nM affinity; thus, CRAC peptide may be useful in the treatment of diseases associated with high cholesterol. The use of drug ligands for this domain could also control cholesterol-lipoprotein interactions in the blood.

Described herein are experiments to identify and design classes of CRAC ligands, including, but not limited to, a) steroid substituted structures; b) synthetic peptides; and c) steroid/peptidomimetic small molecules. Also described herein are biological assays on binding and steroidogenesis modulation. Based on the results of the assays and with the aid of molecular

modeling, further rounds of chemical modification to optimize activity and/or selectivity can be conducted.

Protein import. The role of PBR in mitochondrial protein processing was first suggested by studies showing that PBR drug ligands enhanced mitochondrial processing of the manganese-dependent superoxide dismutase (Wright and Reichenbecher, 1999). These findings were more recently confirmed by the observation that the presence of PBR in OMM functions in the import of the steroidogenic acute regulatory (StAR) protein into mitochondria (Hauet et al., 2005), which regulates of steroid synthesis.

Porphyrin transport and heme biosynthesis. Dicarboxylic porphyrins were found to bind to PBR, which also has been shown to be involved in mammalian tetrapyrrole metabolism (Taketani et al., 1994). In addition, a tryptophan-rich sensory transducer of oxygen (TspO, also called Crtk) homologous to the mammalian PBR was found to regulate the photosynthetic membrane complex formation in *Rhodobacter sphaeroides* and to be involved in *Rhodobacter* transport of porphyrin intermediates (Yeliseev and Kaplan, 2000).

A relationship between the PBR and the heme biosynthetic pathway was also suggested by the interference exerted by methylmercury in PBR-mediated heme biosynthesis (O'Hara et al., 2002). Despite these major advances in the understanding of PBR structure and function, precisely how PBR acts in binding and transfer of cholesterol and tetrapyrrole is still unclear.

Ion transport. PBR coupled cytosolic calcium transport in cells of the gastric mucosa has a potential protective effect linked to $Ca^{2+}$-dependent $Cl^-$ secretion (Ostuni et al., 2004).

Immunomodulation. PBR modulates the function of immunocompetent cells and its expression has also been linked to the inflammatory responses occurring after ischemic-reperfusion injury in an autotransplant pig model (Gavish et al., 1999; Faure et al., 2003).

Cellular respiration and involvement in oxidative processes. PBR drug ligands increase respiratory state IV and decrease respiratory state III rates (Gavish et al., 1999; Lacapere and Papadopoulos, 2003; Larcher et al., 1989). Activation of PBR results in a significant decrease in the respiratory control ratio. The PBR ligands PK 11195 and Ro5-4864 were also found to affect oxygen consumption of mouse C-1300 neuroblastoma cells and of rat brain

cortex mitochondria (Larcher et al., 1989). In addition, exposure of neuronal cells to PBR ligands *in vitro* generates oxygen-free radicals in a process likely involving the MPTP (Jayakumar et al., 2002). PK 11195 has been shown to modulate oxygen-mediated changes in nuclear respiratory factor-1 (Nrf1) expression and block hypoxia- and 2'-deoxyadenosine-induced ocular malformations during embryonic development (O'Hara et al, 2003; Charlap et al., 2003). These findings implicate PBR sites as potential mediators of developmental stress response pathways in the early embryo.

PBR in experimental models and human disease

PBR basal expression is upregulated in a number of neuropathologies, including gliomas (Cornu et al., 1992; Miettinen et al., 1995), neurodegenerative disorders such as Alzheimer's disease (Owen et al., 1983; McGeer et al., 1988; Diorio et al., 1991), as well as in various forms of brain injury and inflammation (Wilms et al., 2003; Chen et al., 2004) induced by neurotoxins such as domoic acid (Kuhlmann and Guilarte, 1997), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Kuhlmann and Guilarte, 1999), trimethyltin (Kuhlmann and Guilarte, 2000) or cuprizone (Chen et al., 2004). A typical feature of neurodegenerative diseases as well as brain injury is activation of microglia and inflammation, leading to gliosis. Considering the high level of expression of PBR in glia as well as the increased proliferation of glia in gliosis, PBR could serve as a diagnostic for the progression of the injury. Indeed, in addition to the diseases and injuries identified above, increased PBR levels have been observed in multiple sclerosis (Vowinckel et al., 1997), Parkinson's and Huntington's diseases (McGeer et al., 1988; Schoemaker et al., 1982; Owen et al., 1983), and epilepsy (Nadler, 1981; MacGregor et al., 1998; Veenman et al., 2002). Increased PBR levels in astroglia have also been associated with hepatic encephalopathy, a condition where a role of ammonia-induced dysfunction of astrocytes has been proposed (Norenberg, 1998). PBR has also been implicated in peripheral nerve degeneration and regeneration (Lacor et al., 1999).

Psychiatric disorders. PBR appears to be involved in the regulation of major stress systems (Ducis et al., 1990), and PBR ligands may exert antianxiety effects via newly synthesized neurosteroids (Papadopoulos et al., 2006). PBR ligand binding induces cholesterol transport into mitochondria and steroid

formation by glia cells leading to the formation of pregnane neurosteroids, such as allopregnanolone and pregnanolone, which positively modulate the functions of the GABA(A) receptor and have anxiolytic effects. This has been shown in animal models of anxiety (Papadopoulos et al., 2006). PBR ligands could potentially prevent psychiatric disorders arising from stress-induced imbalance of CNS function.

Reduced binding levels to PBR have been detected in platelets of patients with anxiety (which were subsequently increased after treatment with diazepam) and in patients with posttraumatic stress disorder. Similarly reduced density of PBR has been observed in soldiers subjected to repeated stress exercises or during war (Gavish et al., 1999). Lower levels of binding to PBR have also been detected in suicidal adolescents (Soreni et al., 1999); however, alterations of PBR do not appear to be significant in patients with obsessive-compulsive disorder or major depression (Gavish et al., 1999).

It has been suggested that diazepam binding inhibitor may modulate schizophrenic symptoms (van Kammen et al., 1993). Decreased density of PBR was observed initially in patients with schizophrenia treated with neuroleptics as compared with untreated patients and normal controls (Gavish et al., 1999). Subsequent studies have demonstrated a significant negative correlation between PBR density in platelets and scores for aggressive behavior, hostility, and anxiety in aggressive patients with schizophrenia (Ritsner et al., 2003).

Cancer. PBR ligands have also been shown to induce cell cycle arrest and apoptosis of human colorectal carcinoma cell lines *in vitro* (Maaser et al., 2001). There are increased levels of PBR in human colon carcinoma tissues as compared with normal human colon tissues (Gavish et al., 1999). Recent studies found that overexpression of PBR in patients with stage III colorectal cancer was an independent prognostic factor and correlated with a poor outcome and reduced survival (Maaser et al., 2002). In addition, correlation between PBR overexpression and progression of breast, colorectal, and prostate cancers has been shown (Han et al., 2003; Galiegue et al., 2004). It must be noted, however, that other factors such as the tissue of origin, may influence the role of PBR in cell proliferation (Kletsas et al., 2004). These observations indicate that PBR could be a target for developing anti-cancer treatments.

A relationship between expression of PBR, its nuclear localization, and its role mediating the transport of cholesterol and proliferation of breast cancer cells with an aggressive phenotype has been reported (Li et al., 2001). Downregulation of PBR using antisense techniques resulted in increased tumorigenicity *in vitro* and *in vivo* of Leydig tumor cells, supporting the hypothesis that deregulation of PBR expression or function may contribute to cellular changes during tumor progression (Weisinger et al, 2004). Cancer cells have increased metabolic requirements due in part to increased proliferation, and PBR-mediated changes in mitochondrial lipid metabolism or respiratory balance leading to mitochondrial biogenesis have been suggested (Ravagnan et al., 1999).

The present invention provides methods to use Structure Based Drug Design (SBDD) studies to identify peptide and substituted steroid compounds that can inhibit the activity of a CRAC domain, and that can control steroid biosynthesis. The identified high binding affinity compounds are then further tested for biological activity. Inhibition of PBR function by the methods and compositions described herein provides a powerful therapeutic for numerous diseases and conditions.

**III. StAR**

Steroidogenic acute regulatory protein (StAR) regulates the limiting step in steroid hormone production in response to hormonal stimuli and transfers cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane (Granot et al., 2002; Soccio and Breslow, 2004; Stocco, 2001). The exact mechanism by which StAR mediates cholesterol transfer in the mitochondria has not been fully characterized; however, there is evidence that StAR activity is mediated by the PBR (Hauet, et al. 2005). Additionally, the tertiary structure of the START (steroidogenic acute regulatory protein (StAR)-related lipid transfer) domain of a StAR homolog has been solved, and identification of a cholesterol-binding hydrophobic tunnel within this domain indicates that StAR acts as a cholesterol-shuttling protein (Soccio and Breslow, 2003).

The StAR family was initially described as a rapidly-induced group of 37-, 32-, and 30-kDa phosphoproteins in ACTH-treated rat and mouse adrenocortical cells, and in LH-treated rat corpus luteum cells and mouse Leydig

tumor cells (Stocco and Clark, 1996). StAR is formed as a 37-kDa cytosolic protein containing an N-terminal mitochondrial signal sequence (Clark et al., 1994), which rapidly transports the protein into mitochondria where it is cleaved, generating the 32-kDa and 30-kDa intramitochondrial forms (Strauss et al., 1999). This protein processing is believed to be responsible for cholesterol transport across the mitochondrial membranes (Clark et al., 1994).

De novo synthesis of StAR parallels the maximal adrenal and gonadal steroid synthesis in response to trophic hormones, and only the newly synthesized 37-kDa StAR is functional and exhibits high activity (Artemenko et al., 2001). Expression of 37-kDa StAR precursor in the absence of hormones induced a two- to three-fold increase in progesterone production by MA-10 cells and isolated mitochondria (Stocco, 1996; Clark et al., 1995). Additionally, recombinant StAR protein, when added directly to purified mitochondria, was shown to increase pregnenolone production (Romanowski et al., 2002). Further studies indicated that there was no need for StAR to enter the mitochondria to exert its steroidogenic function (Arakane et al., 1996; Bose et al., 2002). Thus, it appears that StAR promotes cholesterol transfer as a result of its direct interaction with the outer surface of the mitochondria.

The START (steroidogenic acute regulatory protein (StAR)-related lipid transfer) domain is a protein domain spanning approximately 210 residues. In humans, START domains are found in fifteen distinct proteins, and are classified into six subfamilies: STARD1/StAR group, STARD4 group, STARD2/PCTP group, RhoGAP START group, Thioesterase START group and STARD9 (Alpy, 2005). The STARD1/StAR subfamily consists of STARD3/MLN64 and STARD1/StAR; the STARD4 subfamily consists of STARD4, STARD5, and STARD6; the STARD2/PCTP subfamily consists of STARD11/CERT, STARD10, STARD7, and STARD2/PCTP; the RhoGAP START subfamily consists of STARD12/DLC-1, STARD13/DLC-2, and STARD8; the thioesterase START subfamily consists of STARD15/CACH and STARD14/BFIT; and the STARD9 subfamily consists of STARD9. However, so far only three crystal structures (STARD2/PCTP, STARD3/MLN64 and STARD4) have been solved. These structures reveal a conserved helix-grip fold that forms a wide tunnel to accommodate the hydrophobic lipid (Ponting and Aravind, 1999; Tsujishita and Hurley, 2000; Soccio and Breslow, 2003).

In recent years, many studies have demonstrated the role of StAR in developmentally- and hormonally-regulated steroid biosynthesis (Stocco, 2001; Stocco et al., 2005; Hauet et al., 2002). However, cholesterol transfer is only one of the biological processes in which START domain proteins are implicated. StAR and its homolog, the putative cholesterol transport protein metastatic lymph node 64 (MLN-64; King et al., 2004; Lavaque et al., 2006) were recently found in brain, where no acute regulatory mechanism for StAR synthesis and steroid formation has yet been described. This suggests several possibilities: that there is a currently unidentified acute regulation of StAR expression in brain, or that StAR and its homolog exert a function separate and distinct from that in adrenals and gonads, or that these proteins serve to transfer cholesterol across organelles in a hormone-independent manner.

The present invention provides a method to use Structure Based Drug Design (SBDD) studies to identify substituted steroid compounds that can inhibit the activity of a START domain, and that can control steroid biosynthesis. The identified high binding affinity compounds are then further tested for biological activity. The present invention resulted in the identification of a compound which inhibited approximately 87% of StAR activity at low micromolar concentrations, and other compounds that showed moderate activity. Considering the role of StAR in steroidogenesis, the identified compounds are useful in controlling excessive steroid formation, as occurs in certain adrenal or gonadal tumors. Moreover, in view of the frequent overexpression of START domain-containing proteins in cancer cells, these compounds are useful as anti-tumor agents.

The methods and compositions described herein are contemplated to inhibit or reduce the activity of proteins having START domains, including but not limited to STARD1/StAR, STARD2/PCTP, STARD3/MLN64, STARD4, STARD5, STARD6, STARD7, STARD8, STARD9, STARD10, STARD11/CERT, STARD12/DLC-1, STARD13/DLC-2, STARD14/BFIT, and STARD15/CACH.

Definitions

As used herein, a "steroid" refers to a member of a large family of structurally similar lipid substances. Naturally-occurring steroids function as hormonal messengers in the body. Steroid molecules have a basic skeleton

consisting of four interconnected carbon rings. Different classes of steroids have different functions. Pregnenolone, the precusor molecule for all $C_{18}$, $C_{19}$ and $C_{21}$ steroids, is produced directly from cholesterol. Steroids with 21 carbon atoms are known systematically as pregnanes, whereas those containing 19 and 18 carbon atoms are known as androstanes and estranes, respectively. Steroids include, but are not limited to: neurosteroids, sex hormones, steroid hormone intermediates, dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone, pregnenolone sulfate, all $C_{18}$, $C_{19}$ and $C_{21}$ steroids, progesterone, metabolites of progesterone, aldosterone, testosterone, dihydrotestosterone, 3α-hydroxy-5α-pregnane-20-one (allopregnanalone), 5α-pregnan-3 α -ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, and 11-deoxycorticosterone. The term "steroids" also includes metabolites, derivatives, and intermediates of steroids and steroid synthesis.

The term "neuroactive steroid" refers to steroids which, independent of their origin, are capable of modifying neural activities. Neuroactive steroids bind and modulate different types of membrane receptors. The gamma-aminobutyric acid (GABA) and sigma receptor complexes have been the most extensively studied, while glycine-activated chloride channels, nicotinic acetylcholine receptors, voltage-activated calcium channels, are also modulated by neuroactive steroids. Within the glutamate receptor family, N-methyl-d-aspartate (NMDA) receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors are targets for steroid modulation.

As used herein, the term "steroidogenesis" refers to the production or synthesis of steroids by living organisms.

The term "PBR" or "peripheral-type benzodiazepine receptor" refers to a widely distributed transmembrane protein located mainly in the outer mitochondrial membrane that binds with high affinity to drug ligands and cholesterol (Lacapere et al., 2001). PBR is involved in cholesterol transport from outer to inner mitochondrial membrane and subsequent cholesterol-supported steroidogenesis (Python et al., 1993; Tsankova et al., 1995). Multiple other names may be used to refer to this protein, including mitochondrial

benzodiazepine receptor (MBR), mitochondrial diazepam binding inhibitor (DBI) receptor complex (mDRC), PK 11195 binding sites (PKBS), isoquinoline binding protein (IBP), benzodiazepine receptor peripheral (Bzrp), pk18, and Omega3 (ω3) receptor.

The term "StAR" or "steroidogenic acute regulatory protein" refers to a protein that regulates the limiting step in steroid hormone production in response to hormonal stimuli and transfers cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane. StAR is also referred to as STARD1. This protein is a member of a family of proteins, each having a START domain.

As used herein, the term "START domain" or "steroidogenic acute regulatory protein-related lipid transfer domain" is a protein domain spanning approximately 210 residues. In humans, START domains are found in fifteen distinct proteins, and are classified into six subfamilies: STARD1/StAR group, STARD4 group, STARD2/PCTP group, RhoGAP START group, Thioesterase START group and STARD9 (Alpy, 2005). The STARD1/StAR subfamily consists of STARD3/MLN64 and STARD1/StAR; the STARD4 subfamily consists of STARD4, STARD5, and STARD6; the STARD2/PCTP subfamily consists of STARD11/CERT, STARD10, STARD7, and STARD2/PCTP; the RhoGAP START subfamily consists of STARD12/DLC-1, STARD13/DLC-2, and STARD8; the thioesterase START subfamily consists of STARD15/CACH and STARD14/BFIT; and the STARD9 subfamily consists of STARD9.

The term "inhibiting" or "inhibit" refers to interfering with, reducing, eliminating, or stopping a given action.

The term "binding" or "bind" refers to the interaction between two molecules, for example between a protein or peptide and a ligand. Binding is a specific interaction between the two molecules, and occurs at a particular site or domain in the protein molecule. The interaction of most ligands with their binding sites can be characterized in terms of a binding affinity. In general, high affinity ligand binding results from greater intermolecular force between the ligand and protein or peptide, while low affinity ligand binding involves less intermolecular force between the ligand and protein or peptide. Binding can be

reversible or irreversible. Ligands may include proteins, peptides, peptidomimetics, cholesterol, other lipids, and other small molecules.

As used herein, the term "compound" refers to proteins, peptides, peptidomimetics, cholesterol, other lipids, and other small molecules.

The term "isolated" when used in relation to a nucleic acid, peptide, or polypeptide refers to a nucleic acid sequence, peptide or polypeptide that is identified and separated from at least one contaminant nucleic acid, polypeptide or other biological component with which it is ordinarily associated in its natural source. Isolated nucleic acid, peptide or polypeptide is present in a form or setting that is different from that in which it is found in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule may be double-stranded).

The term "polypeptide" and "protein" are used interchangeably herein unless otherwise distinguished, and "peptide" generally refers to a portion of a full-length polypeptide or protein or an amino acid sequence useful to isolate, purify or detect a linked sequence.

"Transfected," "transformed" or "transgenic" is used herein to include any host cell or cell line, which has been altered or augmented by the presence of at least one recombinant DNA sequence. The host cells of the present invention are typically produced by transfection with a DNA sequence in a plasmid expression vector, as an isolated linear DNA sequence, or infection with a recombinant viral vector.

As used herein, "substantially pure" or "purified" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), for example, a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular

species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, inlcuding more than about 85%, about 90%, about 95%, and about 99%. In one embodiment the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the compounds useful in the present invention can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; solvents include ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences (1985), the disclosure of which is hereby incorporated by reference.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the

tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

One diastereomer of a compound disclosed herein may display superior activity compared with the other. When required, separation of the racemic material can be achieved by HPLC using a chiral column or by a resolution using a resolving agent such as camphonic chloride as in Tucker et al. (1994). A chiral compound of Formula I may also be directly synthesized using a chiral catalyst or a chiral ligand, e.g., Huffman et al. (1995).

"Effective amount" is intended to include an amount of a compound useful in the present invention or an amount of the combination of compounds claimed, e.g., to treat or prevent the disease or disorder, or to treat the symptoms of the disease or disorder, in a host. The combination of compounds can be a synergistic combination. Synergy, as described for example by Chou and Talalay (1984), occurs when the effect of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is most clearly demonstrated at suboptimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased activity, or some other beneficial effect of the combination compared with the individual components.

As used herein, "treating" or "treat" includes (i) preventing a pathologic condition from occurring (e.g. prophylaxis); (ii) inhibiting the pathologic condition or arresting its development; (iii) relieving the pathologic condition; and/or diminishing symptoms associated with the pathologic condition.

As used herein, the term "patient" refers to organisms to be treated by the methods of the present invention. Such organisms include, but are not limited to, mammals such as humans. In the context of the invention, the term "subject" generally refers to an individual who will receive or who has received treatment for treatment of the disease or disorder.

"Substituted" is intended to indicate that one or more hydrogens on the atom indicated in the expression using "substituted" is replaced with a selection from the indicated group(s), provided that the indicated atom's normal valency is not exceeded, and that the substitution results in a stable compound. Suitable indicated groups include, e.g., alkyl, alkenyl alkoxy, halo, haloalkyl, hydroxy,

hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$ and/or $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxy. When a substituent is keto (i.e., =O) or thioxo (i.e., =S) group, then 2 hydrogens on the atom are replaced.

"Interrupted" is intended to indicate that in between two or more adjacent carbon atoms, and the hydrogen atoms to which they are attached (e.g., methyl ($CH_3$), methylene ($CH_2$) or methine (CH)), indicated in the expression using "interrupted" is inserted with a selection from the indicated group(s), provided that the each of the indicated atoms' normal valency is not exceeded, and that the interruption results in a stable compound. Such suitable indicated groups include, e.g., non-peroxide oxy (-O-), thio (-S-), carbonyl (-C(=O)-), carboxy (-C(=O)-), imine (C=NH), sulfonyl (SO) or sulfoxide ($SO_2$).

Specific values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents

"Alkyl" refers to a $C_1$-$C_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$),

3-methyl-3-pentyl (-$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (-$C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl (-$CH(CH_3)C(CH_3)_3$.

The alkyl can optionally be substituted with one or more alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$ and/or $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl. The alkyl can optionally be interrupted with one or more non-peroxide oxy (-O-), thio (-S-), carbonyl (-C(=O)-), carboxy (-C(=O)O-), sulfonyl (SO) or sulfoxide ($SO_2$). Additionally, the alkyl can optionally be at least partially unsaturated, thereby providing an alkenyl.

"Alkenyl" refers to a $C_2$-$C_{18}$ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, $sp^2$ double bond. Examples include, but are not limited to: ethylene or vinyl (-$CH=CH_2$), allyl (-$CH_2CH=CH_2$), cyclopentenyl (-$C_5H_7$), and 5-hexenyl (-$CH_2$ $CH_2CH_2CH_2CH=CH_2$).

The alkenyl can optionally be substituted with one or more alkyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$ and/or $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl. Additionally, the alkenyl can optionally be interrupted with one or more non-peroxide oxy (-O-), thio (-S-), carbonyl (-C(=O)-), carboxy (-C(=O)O-), sulfonyl (SO) or sulfoxide ($SO_2$).

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-$CH_2$-) 1,2-ethyl (-$CH_2CH_2$-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

The alkylene can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$ and/or $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl. Additionally, the alkylene can optionally be interrupted with one or more non-peroxide oxy (-O-), thio (-S-), carbonyl (-C(=O)-), carboxy (-C(=O)O-), sulfonyl (SO), or sulfoxide ($SO_2$). Moreover, the alkylene can optionally be at least partially unsaturated, thereby providing an alkenylene.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

The alkenylene can optionally be substituted with one or more alkyl, alkenyl alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$, and/or $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, alkenyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl. Additionally, The alkenylene can optionally be interrupted with one or more non-peroxide oxy (-O-), thio (-S-), carbonyl (-C(=O)-), carboxy (-C(=O)O-), sulfonyl (SO), or sulfoxide ($SO_2$).

The term "alkoxy" refers to the groups alkyl-O-, where alkyl is defined herein. Alkoxy groups include, e.g., methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

The alkoxy can optionally be substituted with one or more alkyl halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio,

alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$, and $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings, wherein at least one ring is aromatic (e.g., naphthyl, dihydrophenanthrenyl, fluorenyl, or anthryl). Aryls include phenyl, naphthyl, and the like.

The aryl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, heteroaryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$, and $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The cycloalkyl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, heterocycle, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$, and $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl or hydroxyl. .

The cycloalkyl can optionally be at least partially unsaturated, thereby providing a cycloalkenyl.

The term "halo" refers to fluoro, chloro, bromo, and iodo. Similarly, the term "halogen" refers to fluorine, chlorine, bromine, and iodine.

"Haloalkyl" refers to alkyl as defined herein substituted by 1-4 halo groups as defined herein, which may be the same or different. Representative haloalkyl groups include, by way of example, trifluoromethyl, 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, 3-bromo-6-chloroheptyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic, bicyclic, or tricyclic ring system containing one, two, or three aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and which can be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents, like halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include, but are not limited to, 2*H*-pyrrolyl, 3*H*-indolyl, 4*H*-quinolizinyl, 4n*H*-carbazolyl, acridinyl, benzo[*b*]thienyl, benzothiazolyl, β-carbolinyl, carbazolyl, chromenyl, cinnaolinyl, dibenzo[b,d]furanyl, furazanyl, furyl, imidazolyl, imidizolyl, indazolyl, indolisinyl, indolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthyridinyl, naptho[2,3-*b*], oxazolyl, perimidinyl, phenanthridinyl, phenanthrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, thiadiazolyl, thianthrenyl, thiazolyl, thienyl, triazolyl, and xanthenyl. In one embodiment the term "heteroaryl" denotes a monocyclic aromatic ring containing five or six ring atoms containing carbon and 1, 2, 3, or 4 heteroatoms independently selected from the group non-peroxide oxygen, sulfur, and N(Z) wherein Z is absent or is H, O, alkyl, phenyl, or benzyl. In another embodiment heteroaryl denotes an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, or tetramethylene diradical thereto.

The heteroaryl can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heterocycle, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$, and $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl.

The term "heterocycle" refers to a saturated or partially unsaturated ring system, containing at least one heteroatom selected from the group oxygen, nitrogen, and sulfur, and optionally substituted with alkyl or $C(=O)OR^b$, wherein

$R^b$ is hydrogen or alkyl. Typically heterocycle is a monocyclic, bicyclic, or tricyclic group containing one or more heteroatoms selected from the group oxygen, nitrogen, and sulfur. A heterocycle group also can contain an oxo group (=O) attached to the ring. Non-limiting examples of heterocycle groups include 1,3-dihydrobenzofuran, 1,3-dioxolane, 1,4-dioxane, 1,4-dithiane, 2*H*-pyran, 2-pyrazoline, 4*H*-pyran, chromanyl, imidazolidinyl, imidazolinyl, indolinyl, isochromanyl, isoindolinyl, morpholine, piperazinyl, piperidine, piperidyl, pyrazolidine, pyrazolidinyl, pyrazolinyl, pyrrolidine, pyrroline, quinuclidine, and thiomorpholine.

The heterocycle can optionally be substituted with one or more alkyl, alkenyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, imino, alkylamino, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl, cyano, $NR^xR^y$ and $COOR^x$, wherein each $R^x$ and $R^y$ are independently H, alkyl, aryl, heteroaryl, heterocycle, cycloalkyl, or hydroxyl.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles. In one specific embodiment of the invention, the nitrogen heterocycle can be 3-methyl-5,6-dihydro-4H-pyrazino[3,2,1-jk]carbazol-3-ium iodide.

Another class of heterocyclics is known as "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula $[-(CH_2-)_aA-]$ where a is equal to or greater than 2, and A at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, $[-(CH_2)_3-NH-]_3$, $[-((CH_2)_2-O)_4-((CH_2)_2-NH)_2]$ and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "alkanoyl" refers to C(=O)R, wherein R is an alkyl group as previously defined.

The term "acyloxy" refers to –O-C(=O)R, wherein R is an alkyl group as previously defined. Examples of acyloxy groups include, but are not limited to, acetoxy, propanoyloxy, butanoyloxy, and pentanoyloxy. Any alkyl group as defined above can be used to form an acyloxy group.

The term "alkoxycarbonyl" refers to C(=O)OR, wherein R is an alkyl group as previously defined.

The term "amino" refers to $-NH_2$, and the term "alkylamino" refers to $-NR_2$, wherein at least one R is alkyl and the second R is alkyl or hydrogen. The term "acylamino" refers to RC(=O)N, wherein R is alkyl or aryl.

The term "imino" refers to –C=NH.

The term "nitro" refers to $-NO_2$.

The term "trifluoromethyl" refers to $-CF_3$.

The term "trifluoromethoxy" refers to $-OCF_3$.

The term "cyano" refers to -CN.

The term "hydroxy" or "hydroxyl" refers to –OH.

The term "oxy" refers to –O-.

The term "thio" refers to –S-.

The term "thioxo" refers to (=S).

The term "keto" refers to (=O).

The compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "fused ring" used herein can be heterocyclic or carbocyclic.

Unless implied or stated to the contrary, the stereochemistry of the ring junctions and the side-chain attachment in the fused ring systems can be either $\alpha$ (alpha), if below the pane of the paper, or $\beta$ (beta) if above the plane of the paper.

The compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

Selected substituents within the compounds described herein are present to a recursive degree. In this context, "recursive substituent" means that a substituent may recite another instance of itself. Because of the recursive nature of such substituents, theoretically, a large number may be present in any given

claim. One of ordinary skill in the art of medicinal chemistry understands that the total number of such substituents is reasonably limited by the desired properties of the compound intended. Such properties include, by of example and not limitation, physical properties such as molecular weight, solubility or log P, application properties such as activity against the intended target, and practical properties such as ease of synthesis.

Recursive substituents are an intended aspect of the invention. One of ordinary skill in the art of medicinal and organic chemistry understands the versatility of such substituents. To the degree that recursive substituents are present in an claim of the invention, the total number will be determined as set forth above.

The compounds described herein can be administered as the parent compound, a pro-drug of the parent compound, or an active metabolite of the parent compound.

"Pro-drugs" are intended to include any covalently bonded substances which release the active parent drug or other formulas or compounds of the present invention *in vivo* when such pro-drug is administered to a mammalian subject. Pro-drugs of a compound of the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation *in vivo*, to the parent compound. Pro-drugs include compounds of the present invention wherein the carbonyl, carboxylic acid, hydroxy or amino group is bonded to any group that, when the pro-drug is administered to a mammalian subject, cleaves to form a free carbonyl, carboxylic acid, hydroxy or amino group. Examples of pro-drugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups in the compounds of the present invention, and the like.

"Metabolite" refers to any substance resulting from biochemical processes by which living cells interact with the active parent drug or other formulas or compounds of the present invention *in vivo*, when such active parent drug or other formulas or compounds of the present are administered to a mammalian subject. Metabolites include products or intermediates from any metabolic pathway.

Nucleotides, Peptides and Polypeptides. Amino acid and nucleic acid sequences as described herein may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence maintains biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences which may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, i.e., introns, which are known to occur within genes. The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably.

The peptides or polypeptides of the invention can be synthesized *in vitro*, e.g., by solid phase peptide synthetic method or by recombinant DNA approaches. The solid phase peptide synthetic method is an established and widely used method. These polypeptides can be further purified by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on an anion-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; or ligand affinity chromatography.

Chemically modified derivatives of a given peptide or polypeptide can be readily prepared. For example, amides of the peptides or polypeptides of the present invention may also be prepared by techniques well known in the art for converting a carboxylic acid group or precursor, to an amide. A method for amide formation at the C-terminal carboxyl group is to cleave the peptide, polypeptide, or fusion thereof from a solid support with an appropriate amine, or to cleave in the presence of an alcohol, yielding an ester, followed by aminolysis with the desired amine.

Salts of carboxyl groups of a peptide or polypeptide may be prepared in the usual manner by contacting the peptide, or polypeptide with one or more equivalents of a desired base such as, for example, a metallic hydroxide base, e.g., sodium hydroxide; a metal carbonate or bicarbonate base such as, for

example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like.

Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met (M), ala (A), val (V), leu (L), ile (I);

(2) neutral hydrophilic: cys (C), ser (S), thr (T);

(3) acidic: asp (D), glu (E);

(4) basic: asn (N), gln (Q), his (H), lys (K), arg (R);

(5) residues that influence chain orientation: gly (G), pro (P); and

(6) aromatic; trp (W), tyr (Y), phe (F).

Acid addition salts of the peptide or polypeptide or of amino residues of the peptide or polypeptide may be prepared by contacting the polypeptide or amine with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid. Esters of carboxyl groups of the polypeptides may also be prepared by any of the usual methods known in the art.

The peptides or polypeptides of the invention may be labeled, e.g., with a fluorophore or other detectable moiety, which may facilitate detection of binding. Labels and peptides which may facilitate detection include but are not limited to a nucleic acid molecule, i.e., DNA or RNA, e.g., an oligonucleotide, a protein, e.g., a luminescent protein, a peptide, for instance, an epitope recognized by a ligand, for instance, maltose and maltose binding protein, biotin and avidin or streptavidin and a His tag and a metal, such as cobalt, zinc, nickel or copper, a hapten, e.g., molecules useful to enhance immunogenicity such as keyhole limpet hemacyanin (KLH), cleavable labels, for instance, photocleavable biotin, a fluorophore, a chromophore, and the like.

Preparation of Expression Cassettes. Nucleic acids encoding the peptide or polypeptide inhibitors described herein may be introduced into tissue or cells to inhibit steroidogenesis. To prepare expression cassettes encoding the peptide and polypeptide inhibitors described herein for transformation, the recombinant

DNA sequence or segment may be circular or linear, double-stranded or single-stranded. A DNA sequence which encodes an RNA sequence that is substantially complementary to a mRNA sequence encoding a gene product of interest is typically a "sense" DNA sequence cloned into a cassette in the opposite orientation (i.e., 3N to 5N rather than 5N to 3N). Generally, the DNA sequence or segment is in the form of chimeric DNA, such as plasmid DNA, that can also contain coding regions flanked by control sequences which promote the expression of the DNA in a cell. As used herein, "chimeric" means that a vector comprises DNA from at least two different species, or comprises DNA from the same species, which is linked or associated in a manner which does not occur in the "native" or wild-type of the species.

Aside from DNA sequences that serve as transcription units, or portions thereof, a portion of the DNA may be untranscribed, serving a regulatory or a structural function. For example, the DNA may itself comprise a promoter that is active in eukaryotic cells, e.g., mammalian cells, or in certain cell types, or may utilize a promoter already present in the genome that is the transformation target of the lymphotrophic virus. Such promoters include the CMV promoter, as well as the SV40 late promoter and retroviral LTRs (long terminal repeat elements), although many other promoter elements well known to the art may be employed, e.g., the MMTV, RSV, MLV or HIV LTR in the practice of the invention.

Other elements functional in the host cells, such as introns, enhancers, polyadenylation sequences and the like, may also be a part of the recombinant DNA. Such elements may or may not be necessary for the function of the DNA, but may provide improved expression of the DNA by affecting transcription, stability of the mRNA, or the like. Such elements may be included in the DNA as desired to obtain the optimal performance of the transforming DNA in the cell.

The recombinant DNA to be introduced into the cells may contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of transformed cells from the population of cells sought to be transformed. Alternatively, the selectable marker may be carried on a separate piece of DNA and used in a co-transformation procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory

sequences to enable expression in the host cells. Useful selectable markers are well known in the art and include, for example, antibiotic and herbicide-resistance genes, such as *neo, hpt, dhfr, bar, aroA, puro, hyg, dapA* and the like. See also, the genes listed on Table 1 of Lundquist et al. (U.S. Patent No. 5,848,956).

Reporter genes are used for identifying potentially transformed cells and for evaluating the functionality of regulatory sequences. Reporter genes which encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene which is not present in or expressed by the recipient organism or tissue and which encodes a protein whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Exemplary reporter genes include the chloramphenicol acetyl transferase gene (cat) from Tn9 of *E. coli*, the beta-glucuronidase gene (gus) of the *uidA* locus of *E. coli*, the green, red, or blue fluorescent protein gene, and the luciferase gene. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

The general methods for constructing recombinant DNA which can transform target cells are well known to those skilled in the art, and the same compositions and methods of construction may be utilized to produce the DNA useful herein.

The recombinant DNA can be readily introduced into the host cells, e.g., mammalian, bacterial, yeast or insect cells, or prokaryotic cells, by transfection with an expression vector comprising the recombinant DNA by any procedure useful for the introduction into a particular cell, e.g., physical or biological methods, to yield a transformed (transgenic) cell having the recombinant DNA so that the DNA sequence of interest is expressed by the host cell. In one embodiment, the recombinant DNA is stably integrated into the genome of the cell.

Physical methods to introduce a recombinant DNA into a host cell include calcium-mediated methods, lipofection, particle bombardment, microinjection, electroporation, and the like. Biological methods to introduce the DNA of interest into a host cell include the use of DNA and RNA viral vectors. Viral vectors, e.g., retroviral, lentiviral, or adenoviral vectors, have become a widely used method for inserting genes into eukaryotic cells, such as

mammalian, e.g., human cells. Other viral vectors can be derived from poxviruses, e.g., vaccinia viruses, herpes viruses, adenoviruses, adeno-associated viruses, baculoviruses, and the like.

To confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, molecular biological assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; biochemical assays, such as detecting the presence or absence of a particular gene product, e.g., by immunological means (ELISAs and Western blots) or by other molecular assays.

To detect and quantitate RNA produced from introduced recombinant DNA segments, RT-PCR may be employed. In this application of PCR, it is first necessary to reverse transcribe RNA into DNA, using enzymes such as reverse transcriptase, and then through the use of conventional PCR techniques amplify the DNA. In most instances PCR techniques, while useful, will not demonstrate integrity of the RNA product. Further information about the nature of the RNA product may be obtained by Northern blotting. This technique demonstrates the presence of an RNA species and gives information about the integrity of that RNA. The presence or absence of an RNA species can also be determined using dot or slot blot Northern hybridizations. These techniques are modifications of Northern blotting and only demonstrate the presence or absence of an RNA species.

While Southern blotting and PCR may be used to detect the recombinant DNA segment in question, they do not provide information as to whether the recombinant DNA segment is being expressed. Expression may be evaluated by specifically identifying the peptide products of the introduced DNA sequences or evaluating the phenotypic changes brought about by the expression of the introduced DNA segment in the host cell.

Formulations and Dosages

The agents of the invention can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

The agents may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an

assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral administration, the active agent may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active agent. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active agent in such useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active agent may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active agent or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, an isotonic agent, for example, sugars, buffers or sodium chloride can be included. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active agent in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, vacuum drying and the freeze drying techniques can be employed, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the agents may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and

additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Useful dosages of the agents can be determined by comparing their *in vitro* activity and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the agent in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the agent, or an active salt or derivative thereof, required for use alone or with other agents will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

The agent may be conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

In general, however, a suitable dose may be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The active ingredient may be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a

bolus containing about 1-100 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The invention will be further described by the following non-limiting examples.

**Example 1**

**Inhibitors of PBR at the CRAC domain**

**Materials and Methods**

Software: MODELLER 6v2 (Sali et al., 1995) was used to build the homology model of CRAC. UNITY 4.3 (Unity UNITY®; 4.3 ed.; Tripos Inc.: St. Louis. USA) module of Tripos software was used for the pharmacophore searches (Martin,1992) and conformational search and molecular docking calculations have been carried out with SYBYL 7.0 (Sybyl7.0 Tripos Inc.: St. Louis. USA). Using ESFF force field of Insight 2000 (Discover, module; Accelrys Inc.: San Diego) programs, the ligand and protein complexes were energy minimized with 5000 iterations of the conjugate gradient algorithm such that the square root of the average magnitude of the force was less than or equal to 0.01 kcal/(molÅ). All the molecular modeling calculations were carried out on Silicon Graphics Octance 2 workstation.

Biological activity studies: [1,2.6,7-3H(N)] progesterone (specific activity, 92 Ci/mmol) was obtained from NEN Life Science Products (Boston, MA). Anti-progesterone antisera was purchased from ICN Pharmaceuticals (Costa Mesa, CA).

Progesterone Biosynthesis: MA-10 mouse Leydig tumor cells were plated into 96-well plates at the density of $2.5 \times 10^4$ cells per well for overnight. The cells were washed with serum-free medium and treated with compounds at

different doses for 3 hours at 37°C, then 1 mM dibutyryl cAMP (Aldrich Advancing Science, Milwaukee, WI) was added per well for additional 2 hours incubation at 37°C. The culture media were collected and tested for progesterone production by radioimmunoassay (RIA) using anti-progesterone antisera, following the conditions recommended by the manufacturer. Progesterone production was normalized by the amount of protein in each well.

**Results and Discussion**

To obtain highly selective and potent PBR inhibitors at Cholesterol Recognition Amino acid Consensus (CRAC; Li et al, 2001; Lacapere and Papadopoulos, 2003) site, a structure-based pharmacophore design consisting of the following steps was performed: (1) Building the homology models of CRAC peptide and molecular minimization studies (2) Identification of pharmacophores at the cholesterol binding region (3) Lead generation of a new classes of steroid and small molecule inhibitors by virtual screening of commercially available database compounds and molecular docking methods and (4) Designing novel peptide structures.

Homology Modeling and Molecular Minimization: A structure-based approach to design specific inhibitors for PBR by utilizing homology modeling was undertaken. Homology models have been used successfully to generate new ligands against several different classes of drug targets. The three-dimensional (3D) structure of PBR CRAC peptide is not completely characterized. An NMR representation of the CRAC sequence was recently published, but docking simulation with various ligands allowed the refinement and validation of the 3D structure of the CRAC sequence.

The mouse, rat and human CRAC domain residues 147-158 are:

**Mouse** (147) A T V L N Y Y V W R D N (158) (SEQ ID NO:6)
**Rat** (147) A T M L N Y Y V W R D N (158) (SEQ ID NO:7)
**Human** (147) A T T L N Y C V W R D N (158) (SEQ ID NO:8)

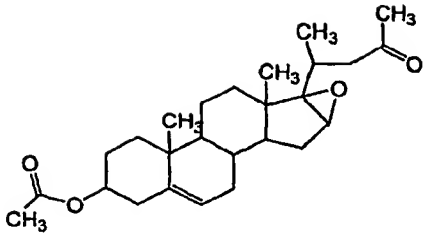
A comparison of the sequence alignment reveals that most of the residues at the cholesterol-binding region are similar (Amri et al., 1998). However, some residues are different (underlined). The input alignment for the MODELLER was obtained with BlastP searches based on the sequence of the mouse, rat and human PBR CRAC peptides, the homology models were built from the crystal

structures of 1A99.pdb and 1P9M.pdb (Li and Papadopoulos, 2005; Dmitry et al., 1998), and these models were used for the structure-based design. Several mutagenesis studies have revealed that the CRAC region of PBR can accommodate cholesterol (Boulanger et al., 2003). Hence, in the instant invention, the cholesterol structure was docked into the active site and the complex was energy minimized. In addition, residues adjacent to the cholesterol binding cleft have also been identified. Interaction of appropriately designed ligands with these sites may lead to increased specificity and potency.

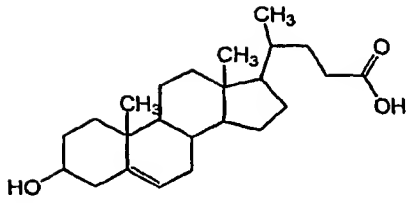
Pharmacophore search: In most cases, methods for structure-based lead generation were based on computational descriptions of a binding site, as in the coordinates of atoms or pharmacophores. In the current study we used a human model of CRAC for the identification of novel PBR inhibitors. The cholesterol binding site of the PBR is a deep cleft formed between Y152 and N156 as shown in Figure 1. The hydrogen bond donors of Y152 and R156 were used for the pharmacophore search. As the hinge region accommodates moderately planar moieties close to the C153 anchor region, the pharmacophore search results were analyzed accordingly. Pharmacophoric templates utilized to conduct a 2D UNITY search of the database are given in Figure 2. To define the pharmacophores, the acceptor (A) and donor (D) sites of the Flex pharmacophores were defined without distance constraint. By using receptor site module, exclusion spheres were defined up to 5Å region from the pharmacophore site to get appropriately docked structures. A database search based on the above pharmacophore generated a set of 700 hits.

Identification of the lead compounds: The 700 compounds were docked into the cholesterol binding site using several molecular docking programs. The docking programs FlexiDock, FlexX, DOCK 5.0, and AutoDock 3.0 were used to rank order the compounds (Jamin et al., 2005; FlexDock 6.9 ed., Tripos Inc.: St. Louis; Kramer et al., 2001; Kuntz, 1992). Using a consensus scoring function, which is a combination of the molecular mechanics energy, surface area, and statistical parameter derived from known ligand-protein x-ray crystal structure complexes (Goodsell and Olson, 1990), 70 compounds were selected. These 70 structures were further viewed on a 3D graphics screen for orientations, binding modes and diversity. In Figure 3, the dotted lines indicate the intermolecular hydrogen bond interactions with Y152 and R156. Structures

that docked with proper orientation and with inter-molecular H-bonding were selected for further rounds of minimization. Finally, the selected best rank compounds with their protein complexes were energy minimized using extensible systematic force field to better fit the ligands into the cholesterol binding pocket. The binding orientation of compound 1 is shown in Figure 3. Based on the above factors, the top ranking steroid and small molecules were selected for biological assays using Leydig MA-10 cells, which produce steroid. The structures of tested compounds are shown below.



Compound 1



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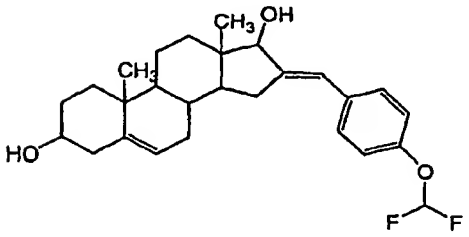

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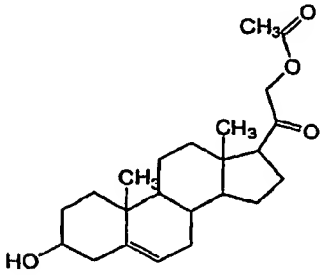

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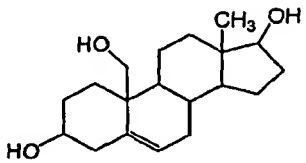
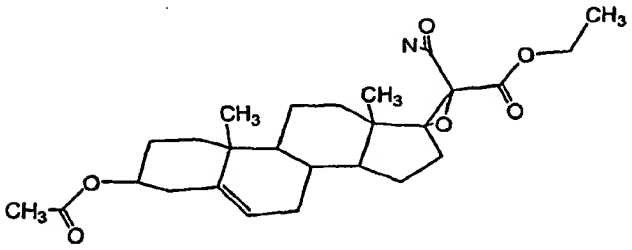
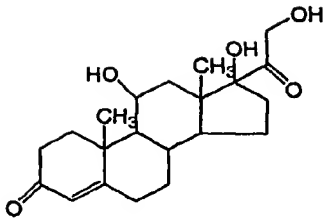


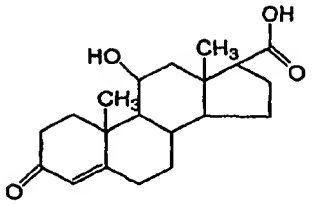


Compound 5

Compound 6




Compound 7

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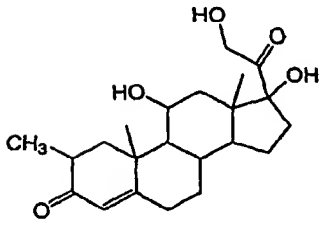


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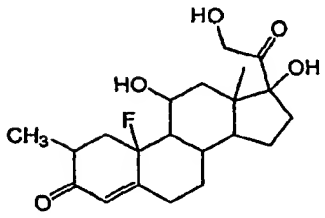


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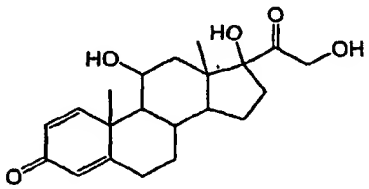


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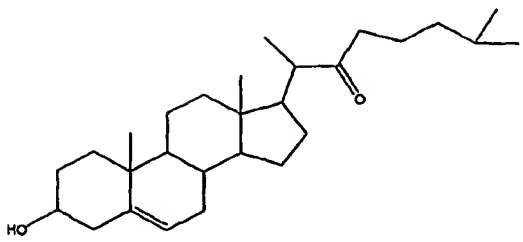

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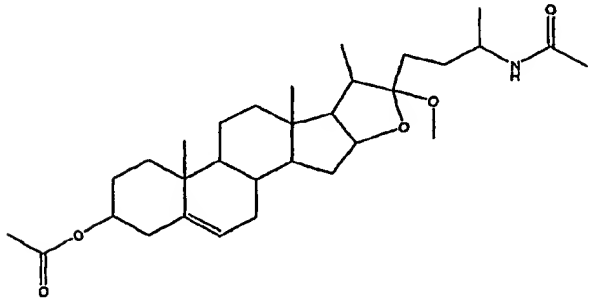

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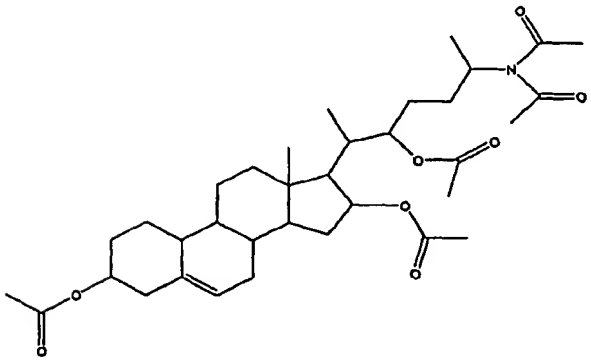

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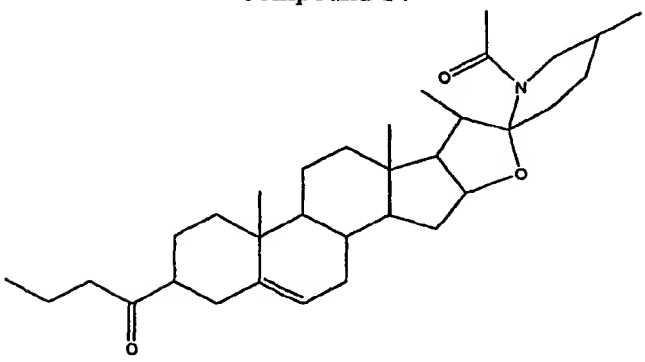
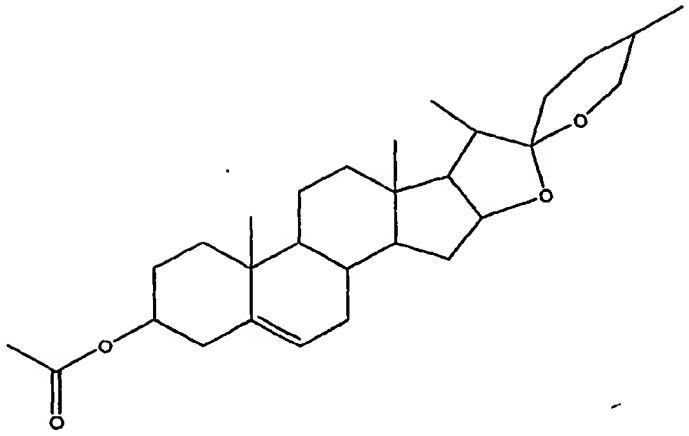
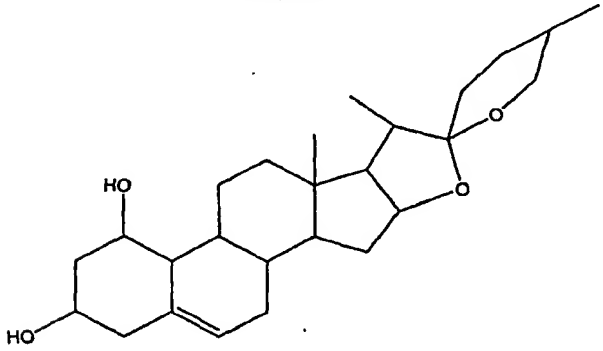


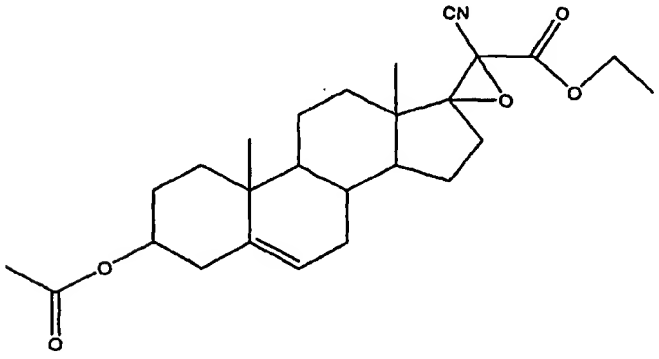
Compound 15



Compound 16




Compound 17

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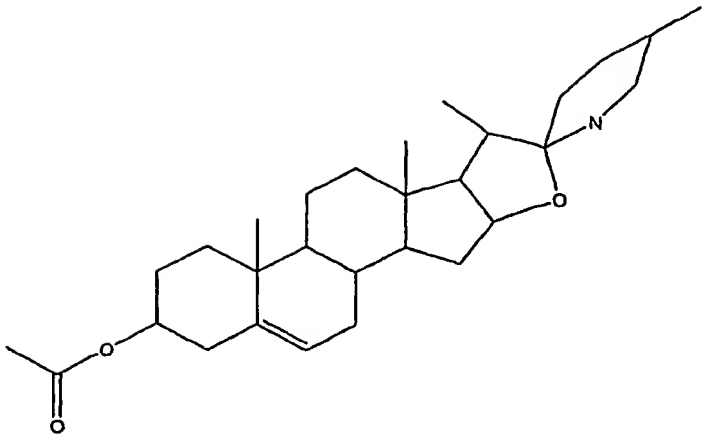


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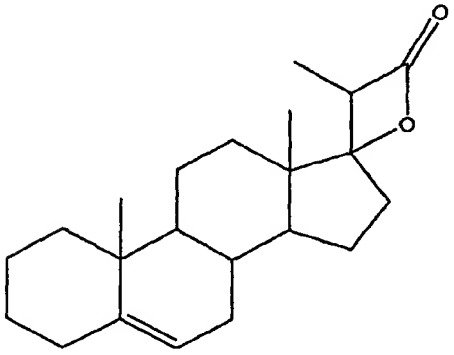


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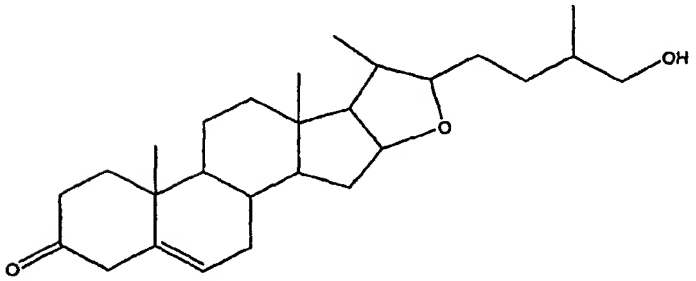


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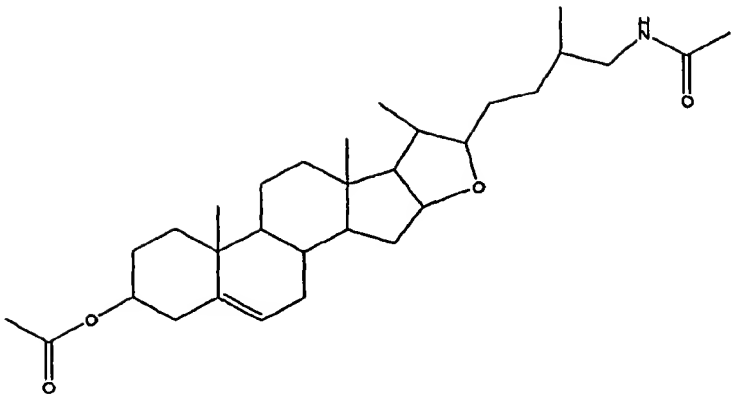
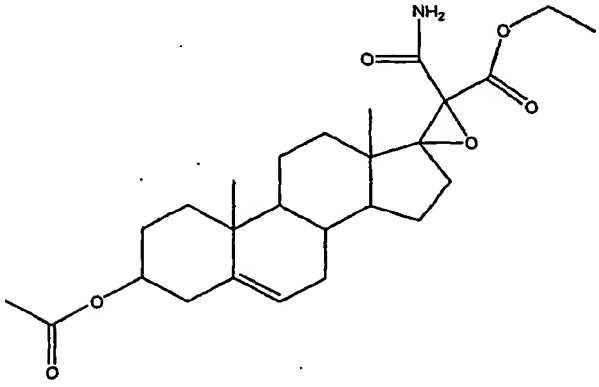


Compound 22



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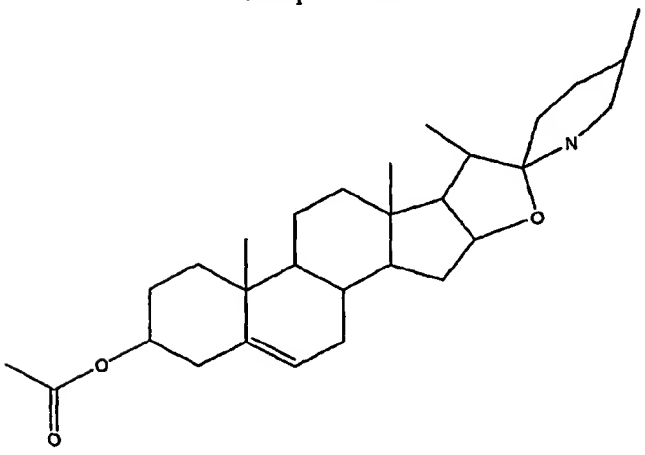

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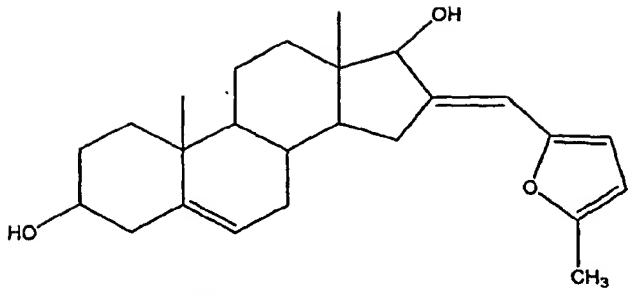


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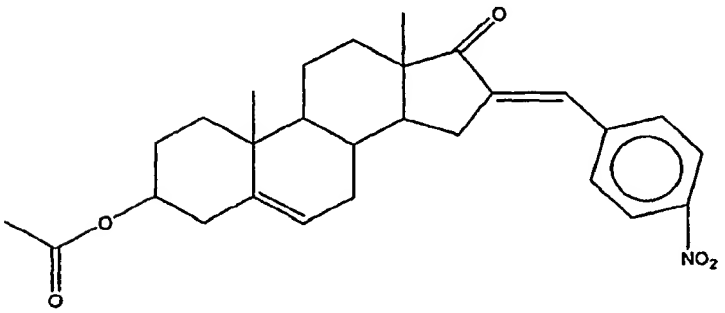


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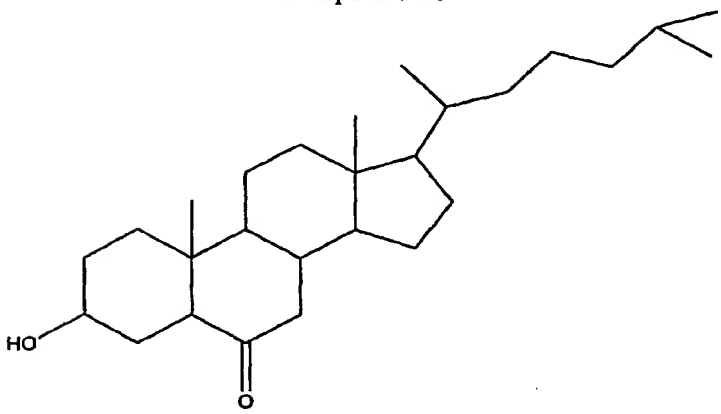


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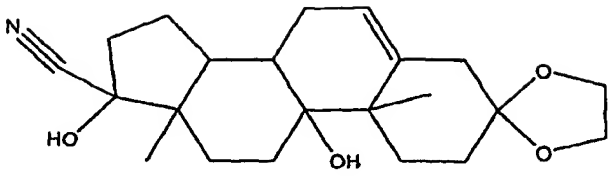
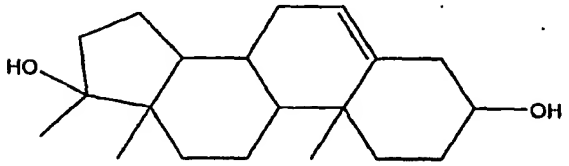



Compound 28

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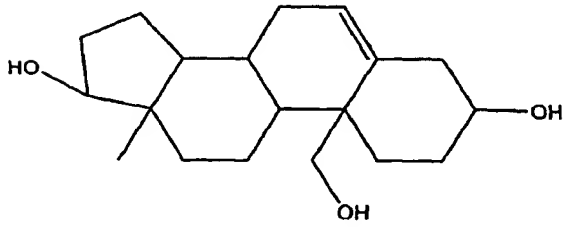
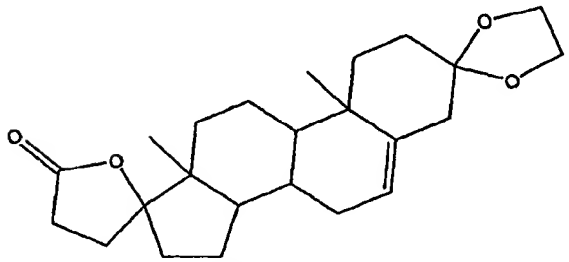



Compound 30

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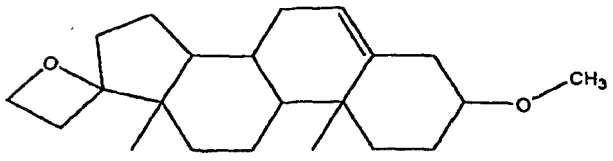


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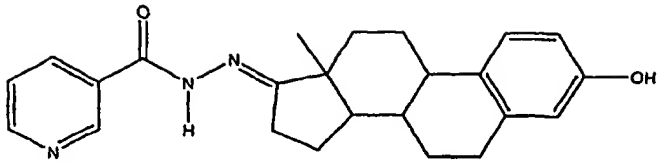


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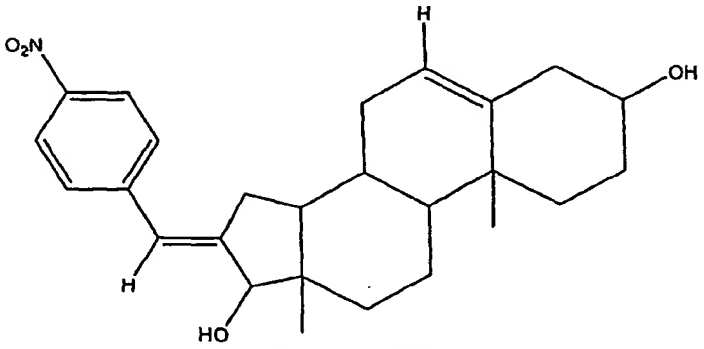


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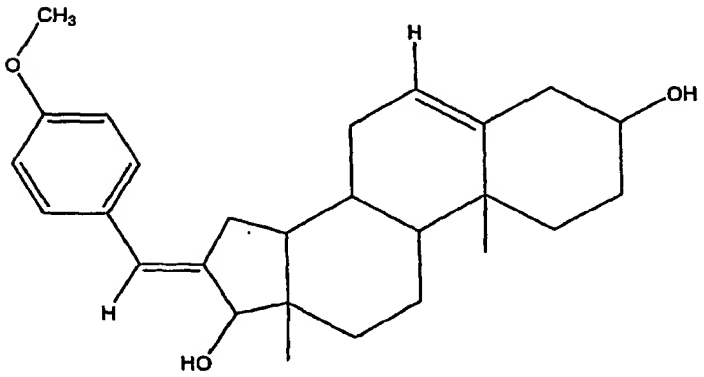
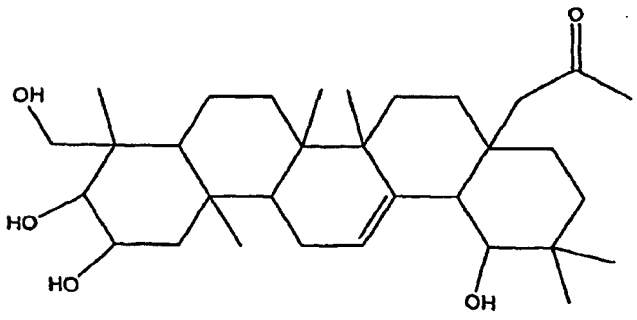



Compound 35




Compound 36

All the compounds tested displayed negative effects on progesterone synthesis, confirming the docking simulation.

Designing new peptide structures: Considering the residues that are involved in cholesterol binding and reported mutational data on CRAC peptide, peptides were built. These structures are developed based on molecular interactions including hydrogen bonding, hydrophobic and other interactions with residues at the interaction site. Using GA-based conformational search, the low energy conformation structure was used for further calculations. These structures were docked at the cholesterol binding site and the ligand-protein complexes were minimized. Based on the binding energies a set of peptides structures was developed. These peptides, their docking scores, and binding energies are shown in Table 1.

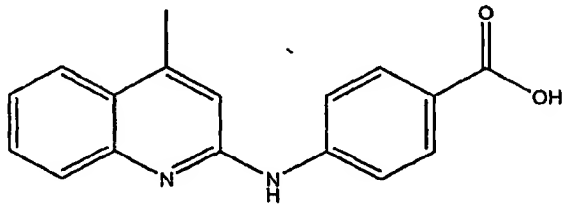
**TABLE 1**

| Peptide | Docking Score | Binding Energy |
|---|---|---|
| DAHAD (SEQ ID NO:9) | -17.40 | -150.00 |
| DRAVH (SEQ ID NO:10) | -25.26 | -150.00 |
| DRVAN (SEQ ID NO:11) | -17.75 | -98.00 |
| FAHAD (SEQ ID NO:12) | -14.24 | -123.00 |
| AGNVD (SEQ ID NO:13) | -23.25 | -200.00 |
| ALNVD (SEQ ID NO:14) | -23.80 | -198.00 |
| AVNLD (SEQ ID NO:15) | -16.22 | -116.00 |
| AVNVD (SEQ ID NO:16) | -17.36 | -159.00 |
| FANAD (SEQ ID NO:17) | -18.00 | -113.00 |
| FANAR (SEQ ID NO:18) | -13.62 | -164.00 |
| FRAVH (SEQ ID NO:19) | -11.14 | -149.00 |

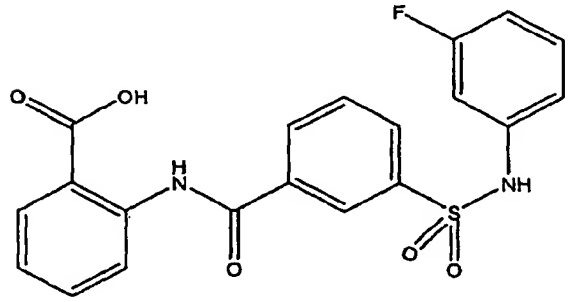
Those of skill in the art will appreciate that the structure of the side chains can be modified and designed to achieve specificity and enhancement of inhibition for PBR activity, depending upon the activity profile of the assayed compounds.

Identification of peptidomimetic structures: To identify peptidomimetic structures, one of the high binding affinity peptide structures underwent pharmacophore searches with distance constraints. The pharmacophores were

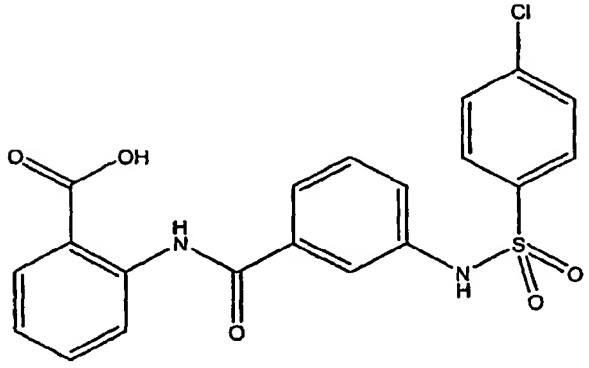
defined as represented in Figure 5 and the structures were identified from various databases using virtual screening methods and are shown below.



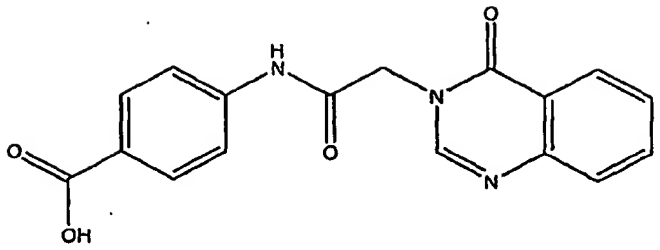

Compound 37



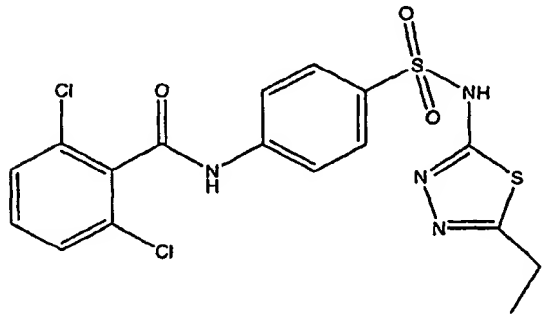

Compound 38



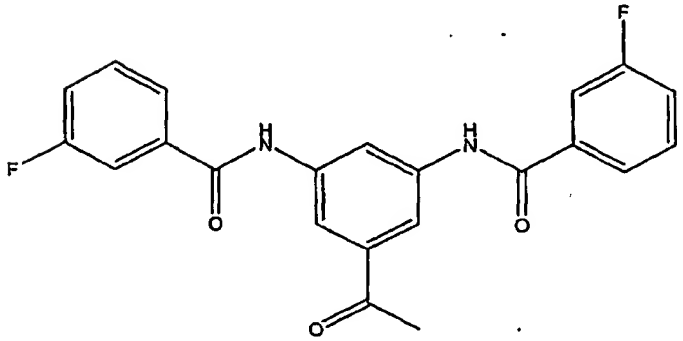

Compound 39



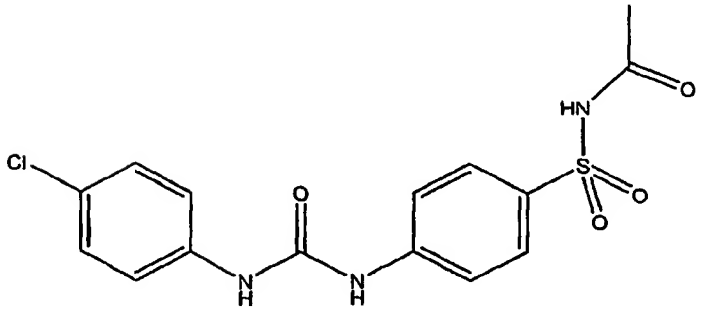
Compound 40



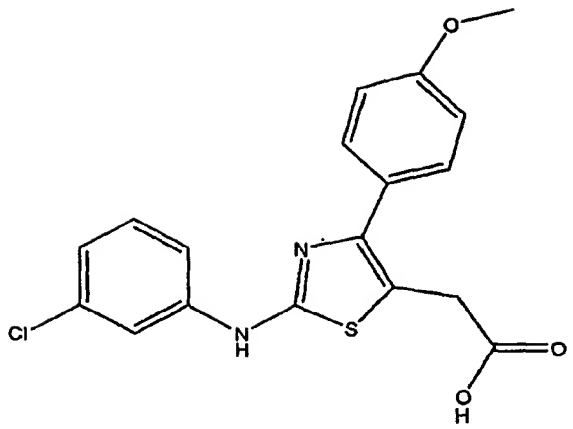
Compound 42



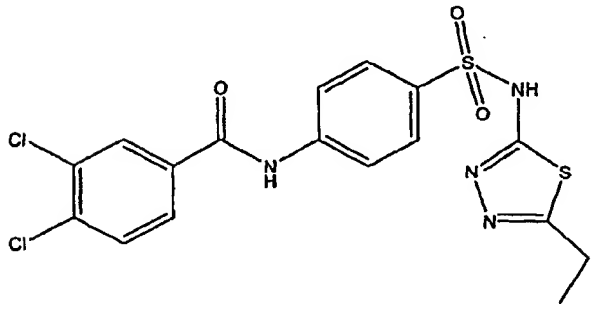
Compound 43



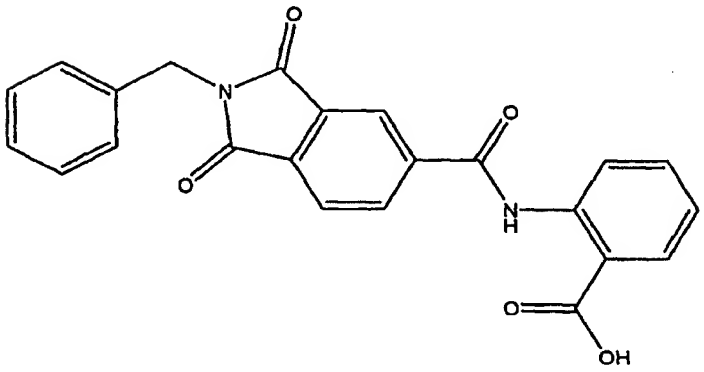
Compound 44



Compound 45



Compound 46



Compound 47

The combined use of different computational approaches allowed the identification of peptide CRAC ligands, the development of a new series of

peptide CRAC ligands, and the development of a new series of peptidomimetic CRAC ligands. Compounds from each of these three classes of ligands received favorable in silico docking scores. Additionally, it was demonstrated that the biological activity of certain compounds can be accurately extrapolated and anticipated by using the 3D conformation of the CRAC sequence provided by the calculation protocol described herein. These computational approaches also demonstrated that the CRAC sequence of PBR represents a good therapeutic target, especially for treating diseases involving a dysregulation of steroid synthesis.

## Example 2

## Inhibitors of StAR at the START domain

### Materials and Methods

Molecular Modeling studies: Homology models were built using the program MODELLER 6v2 (Sali et al., 1995; Fiser et al., 2003; John et al., 2003). Multiple sequence alignments were prepared by using clustalW (Oliver et al., 2005). The input alignment for the Modeler was obtained with BlastP searches based on the sequence of the human StAR and mouse StAR, and the homology models were built from the crystal structures of 1EM2.pdb (STARD3/MLN64; Tsujishita et al., 2000) and 1JSS.pdb (mouse StARD4; Romanowski et al., 2002). Biopolymer module and FlexX programs of SYBYL 7.0 software were used to study the possible binding pockets and calculate the molecular docking scoring functions. The built protein models and the ligand (cholesterol) complexes were energy minimized with Affinity module of INSIGHT II (Accelrys Inc., San Diego, CA).

Biological activity studies: [1,2.6,7-3H(N)] progesterone (specific activity, 92 Ci/mmol) was obtained from NEN Life Science Products (Boston, MA). Anti-progesterone antisera was purchased from ICN Pharmaceuticals (Costa Mesa, CA).

Progesterone Biosynthesis: MA-10 cells were plated into 96-well plates at the density of $2.5 \times 10^4$ cells per well for overnight. The cells were washed with serum-free medium and treated with compounds at different doses for 3 hours at 37°C, then 1 mM dibutyryl cAMP (Aldrich Advancing Science, Milwaukee, WI) was added per well for additional 2 hours incubation at 37°C.

The culture media were collected and tested for progesterone production by radioimmunoassay (RIA) using anti-progesterone antisera, following the conditions recommended by the manufacturer. Progesterone production was normalized by the amount of protein in each well.

Protein Assay: Microgram amounts of protein were measured by using Bio-Rad protein assay dye reagent (Bio-Rad laboratories, Hercules, CA), with bovine serum albumin (ICN Pharmaceuticals) as the standard.

**Results and Discussion**

The crystal structures of StARD2 (Roderick et al., 2002), MLN64/StARD3, and StARD4 have been solved and reported in the RCSB Protein Data Bank. Their structural features are similar but the sequence identity between these polypeptides is low. From the clustalW sequence alignment it was observed that MLN64 and StARD4 have about 35% and about 30% sequence identity, respectively, with StARD1 domain (Figure 6). These two PDB structures were used to build the homology 3D model of human and mouse (H/M) StAR domain. This structure was, in turn, used to identify the probable binding pockets that were used to evaluate the cholesterol/lipid-binding region.

SiteID is a program that provides analysis and visualization tools to identify potential binding sites within or at the surface of macromolecules. It records the properties such as hydrogen bonding character, hydrophobicity, and solvent-accessible surface. Using siteID, maximum size binding pockets were located in all of the three START domain structures (MLN64, StARD4 and H/M StAR). In all the structures, the lipid-binding region is similar. However, when the residues up to 7.0Å residues, which are occupying the lipid-binding region were extracted from the defined pocket, it was found that the surrounding residues are different in MLN64, StARD4 and H/M StAR (see Figure 6). Residues V151, I54, E169, F184, L243, I245, L260, T263 and F267 in HstAR (outlined by black lines) were found to function in lipid binding in all StAR domains (Figure 6). It was observed that except E173/A172 and D266/E265 (underlined in Figure 6), all of the remaining residues are similar in the cholesterol binding region. Hence, in the present molecular docking calculations only the H/M StAR structure was employed.

To optimize the lipid binding pocket and the 3D homology models, molecular mechanics calculations were performed with cholesterol-H/M StAR

complexes using Simulated Annealing (SA) method carried out by Affinity module of InsightII software. Affinity studies were carried out for the representative structure, cholesterol. First, the cholesterol structure was docked into the identified binding site of the H/M StAR homology model and the residues which were close to the site were also allowed to move freely along with internal bonds. Subsequently, the Ligand-Protein (L-P) complex was minimized with 10,000 cycles of minimization steps. Only ten energetically favorable conformations were accepted. Among the ten conformations, the low energy L-P complex was selected for further analysis. From the low energy conformation it is remarkable to note that the orientation of the cholesterol is towards the C-Terminus;, also, the lipid binding tunnel region is observed from N to C-Terminal (Figure 7A).

The optimized H/M StAR structrure was then used in virtual screening to identify small molecular inhibitors for StAR. The present invention permits the virtual screening of compounds from databases of 15 million small molecules and commercially available compounds without any biological input. Hence, a database comprising more than 700 steroid structures was extracted from 15 million structures in sixteen commercially available databases. This dataset of structures was used for pharmacophore and molecular docking studies at the cholesterol/lipid binding site of the H/M StAR domain. Based on the identity of the residues involved in cholesterol binding of the StAR domain, the pharmacophores were defined (Figure 8) in order to determine the specific intermolecular interacting ligands from the steroid database. Among the seven hundred steroid molecules available in the database, approximately 90% of them remained candidates after the pharmacophore search screen. Thus, to obtain those molecules with the best docking capabilities and molecular interactions, FlexX was used to dock the entire steroid database.

Molecular Docking programs such as Dock and FlexX were used to dock all the steroid database structures at the optimized cholesterol-binding site. All docking trials were performed with FlexX at an active site radius of 7.0 Å for all proteins. Using a consensus scoring function (which is a combination of the molecular mechanics energy, surface area, and statistical parameter derived from known ligand-protein x-ray crystal structure complexes), the best-docked structures were chosen for energy minimization using the molecular mechanics

CFF91 forcefield (Accelrys Inc., San Diego, CA) to better fit the ligands into the lipid-binding pocket.

The docking orientation of high binding affinity structure (Str1) and the intermolecular interactions with lipid binding residues (residues outlined by black lines in Figure 6) at binding sites are represented in Figure 7B-C. The hydrogen-bonding interactions of Str1 with V151, E169, T263 (dotted lines in Figure 7C) and hydrophobic interactions with V151, I154, L243, I245 and L260 clearly indicates the binding affinity of the structure. Based on the above factors, compounds 1-28 (see above) were selected for biological activity studies against StAR (Figure 9). From the biological activity studies it was observed that compounds 3 (ID: 5558689), 4 (ID: 7200532) and 5 (ID: 5936472) were likely to possess inhibitory activity against START domains (Figure 10).

With the help of various computational techniques, the human and mouse 3D models of the START domain were developed. Using binding site analysis, the lipid-binding region of the domain was identified and optimized. These models were used for docking members of a steroid database to identify steroidogenesis down-regulating compounds. Based on the binding affinities and intermolecular interactions of the compounds, a set of structures was chosen for further bioactivity studies. Compound 5 induced a roughly 87% inhibition of StAR activity. As would be appreciated by one of skill in the art, the compounds with highest consensus scores and resultant inhibition of steroidogenesis can serve as lead compounds for the design and development of therapeutic agents for the control of steroid biosynthesis in cells in which StAR protein is overexpressed and in disorders where other START domain-containing proteins are overexpressed, as in certain cancers (Alpy et al., 2005).

The present invention provides a method to use Structure Based Drug Design (SBDD) studies to identify substituted steroid compounds that can inhibit the activity of a StAR domain, and that can control steroid biosynthesis. The identified high binding affinity compounds were further tested for biological activity. The present invention resulted in the identification of a compound which inhibited approximately 87% of StAR activity at low micromolar concentrations, and other compounds that showed moderate activity. Considering the role of StAR in steroidogenesis, the identified compounds are useful in controlling excessive steroid formation, as occurs in certain adrenal or

gonadal tumors. Moreover, in view of the frequent overexpression of START domain-containing proteins in cancer cells, these compounds can be used as anti-tumor agents.

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Sybyl7.0 Tripos Inc.: St. Louis. USA
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All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that

certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

**WHAT IS CLAIMED:**

1. A method to inhibit steroidogenesis in a cell, comprising contacting a cell that conducts steroidogenesis with a compound that binds to a START or CRAC domain, wherein the compound is effective to inhibit steroidogenesis in said cell.

2. A method to inhibit steroidogenesis in a subject in need thereof, comprising administering a compound that binds to a START or CRAC domain in an amount effective to inhibit steroidogenesis in said subject.

3. A method to inhibit activity of a START or CRAC domain, comprising contacting a START or CRAC domain with a compound that binds to the START or CRAC domain and thereby inhibits the activity of said START or CRAC domain.

4. A method to inhibit interaction between cholesterol and a CRAC or START domain comprising administering a compound that binds to a CRAC or START domain in an amount effective to inhibit the interaction between said cholesterol and said CRAC or START domain.

5. A method to treat a condition that is characterized by overproduction of a steroid in a subject in need thereof, comprising administering a compound that binds to a START or CRAC domain in an amount effective to treat said condition.

6. The method of claim 1 or 2, wherein the steroidogenesis produces a steroid selected from the group consisting of pregnenolone and progesterone.

7. The method of claim 1 or 2, wherein the steroidogenesis produces a steroid selected from the group consisting of pregnenolone and progesterone metabolites.

8. The method of claim 1 or 2, wherein the steroidogenesis produces a steroid selected from the group consisting of dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone sulfate, aldosterone, testosterone, dihydrotestosterone, 3α-hydroxy-5α-pregnane-20-one (allopregnanalone), 5α-pregnan-3 α-ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, and 11-deoxycorticosterone.

9. The method of claim 1, 2, 3, 4, or 5, wherein the START domain is present in a protein selected from the group consisting of STARD3/MLN64, STARD1/StAR, STARD4, STARD5, STARD6, STARD11/CERT, STARD10, STARD7, STARD2/PCTP, STARD12/DLC-1, STARD13/DLC-2, STARD8, STARD15/CACH, STARD14/BFIT, and STARD9.

10. The method of claim 1, 2, 3, 4, or 5, wherein the CRAC domain is present in peripheral-type benzodiazepine receptor.

11. The method of claim 1, 2, 3, 4, 5, or 9, wherein the START domain comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

12. The method of claim 1, 2, 3, 4, 5, or 10, wherein the CRAC domain comprises a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

13. The method of claim 1, 2, 3, 4, or 5, wherein the compound is a polypeptide selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19.

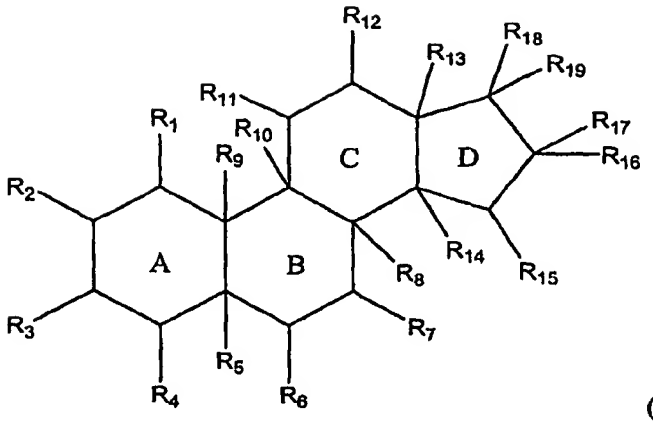
14. The method of claim 5, wherein the steroid is selected from the group consisting of pregnenolone and progesterone.

15. The method of claim 5, wherein the steroid is selected from the group consisting of pregnenolone and progesterone metabolites.

16. The method of claim 5, wherein the steroid is selected from the group consisting of pregnenolone, progesterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone sulfate, aldosterone, testosterone, dihydrotestosterone, 3α-hydroxy-5α-pregnane-20-one (allopregnanalone), 5α-pregnan-3 α-ol-20-one, tetrahydrodeoxycorticosterone (THDOC), 17-OH pregnenolone, 17-OH-progesterone, androstenedione, androstenedione sulfate, estradiol, esterone, cortisol, corticosterone, deoxycorticosterone, 11-deoxycortisol, and 11-deoxycorticosterone.

17. The method of claim 5, wherein the condition is selected from the group consisting of glioma, neurodegenerative disorder, brain injury, brain inflammation, Alzheimer's disease, ischemia-reperfusion injury, epilepsy, affective disorder, fatigue during pregnancy, premenstrual syndrome, postpartum depression, catamenial epilepsy, alcoholism, sleep disorder, memory disorder, premenstrual dysphoric disorder, mood disorder, depressive disorder, anxiety disorder, eating disorder, dementia, stress disorder, aggressiveness, convulsions, pain, neuronal degeneration, neurite outgrowth disorder, synaptogenesis disorder, hyperpituitarism, multiple sclerosis, Parkinson's disease, Huntington's disease, hepatic encephalopathy, peripheral nerve degeneration, adrenal hyperplasia, gonadal hyperplasia, and colon carcinoma.

18. The method of claim 1, 2, 3, 4, or 5, wherein the compound has the structure of Formula (I):




(I)

wherein:

fused rings A, B, C, and D are independently saturated or fully or partially unsaturated; and

$R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ each independently comprises hydrogen, hydroxyl, oxo, halogen, cyano, nitro, carboxy, substituted or unsubstituted amino, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) hydroxyalkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyloxy-($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarboxy-($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{20}$) alkyl, or -$SO_2$A, wherein A comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl;

or $R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ together with the atoms they are attached can form a substituted or unsubstituted carbocyclic or a substituted or unsubstituted heterocyclic ring, wherein the substituted carbocyclic or the substituted heterocyclic ring may be substituted with one or more substituted or unsubstituted carbocyclic rings or one or more substituted or unsubstituted heterocyclic rings;

or $R_{16}$ and $R_{17}$ may be taken together as =C($R_aR_b$), wherein $R_a$ and $R_b$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl;

or $R_{18}$ and $R_{19}$ may be taken together as =N-N($R_cR_d$), wherein $R_c$ and $R_d$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$)

alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, heterocyclylcarbonyl, or ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl; and

$R_5$, $R_8$, $R_9$, $R_{10}$, $R_{13}$, and $R_{14}$ is each independently: deleted when one of fused rings A, B, C, or D is unsaturated so as to complete the valency of the carbon atom at that site, or

each independently comprises hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) hydroxyalkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyloxy-($C_1$-$C_{18}$) alkyl;

or a pharmaceutically acceptable salt thereof.

19. The method of claim 18, wherein

$R_1$ through $R_4$, $R_6$, $R_7$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, $R_{18}$, and $R_{19}$ each independently comprises hydrogen, hydroxyl, oxo, methyl, ethylenedioxy, cyano, $-CH_2OH$, $-OCOCH_3$, $-COCH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2COOH$, $-COCH_2OCOCH_3$, $-COCH_2OH$, $-COOH$, $-CO(CH_3)COCH_2CH_2CH_2CH(CH_3)_2$, or $-OCOCH_3$;

or $R_{16}$ and $R_{19}$ together with the atom or atoms to which they are attached can form a tetrahydrofuran ring;

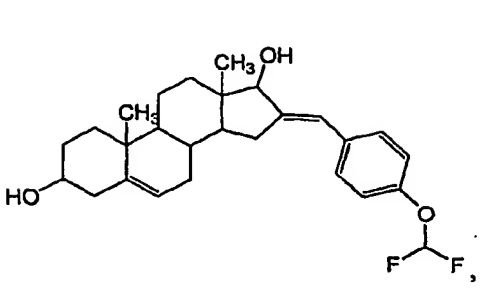
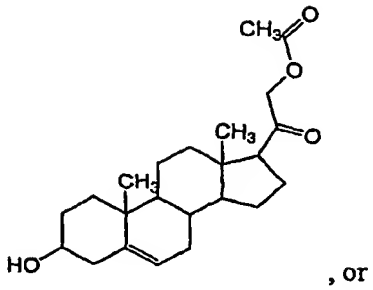
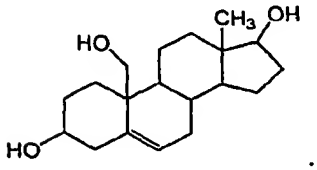
or $R_{16}$ and $R_{17}$ may be taken together as $=C(R_aR_b)$, wherein $R_a$ and $R_b$ each independently comprises hydrogen, p-nitrophenyl, p-methoxyphenyl, or 2-(5-methyl furfuryl); and

or $R_{18}$ and $R_{19}$ may be taken together as $=N\text{-}N(R_cR_d)$, wherein $R_c$ and $R_d$ each independently comprises hydrogen or –C(O)-3-pyridene; and

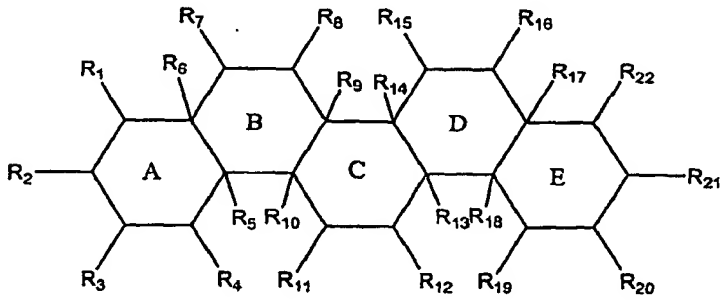
$R_5$, $R_8$, $R_9$, $R_{10}$, $R_{13}$, and $R_{14}$ is each independently: deleted when one of fused rings A, B, C, or D is unsaturated so as to complete the valency of the carbon atom at that site, or

each independently comprises hydrogen, hydroxyl, or methyl.

20. The method of claim 18, wherein the compound is



, or


21. The method of claim 1, 2, 3, 4, or 5, wherein the compound has the structure of Formula (II):


(II)

wherein:

fused rings A, B, C, D, and E are independently saturated or fully or partially unsaturated; and

$R_1$ through $R_4$, $R_6$, $R_7$, $R_8$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{19}$, $R_{20}$, $R_{21}$, and $R_{22}$ each independently comprises hydrogen, hydroxyl, oxo, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, or substituted or unsubstituted ($C_1$-$C_{18}$) hydroxyalkyl;

or $R_5$, $R_6$, $R_9$, $R_{10}$, $R_{13}$, $R_{14}$, $R_{17}$, and $R_{18}$ is each independently: deleted when one of fused rings A, B, C, D, or E is unsaturated so as to complete the valency of the carbon atom at that site, or

each independently comprises hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl;

or a pharmaceutically acceptable salt thereof.

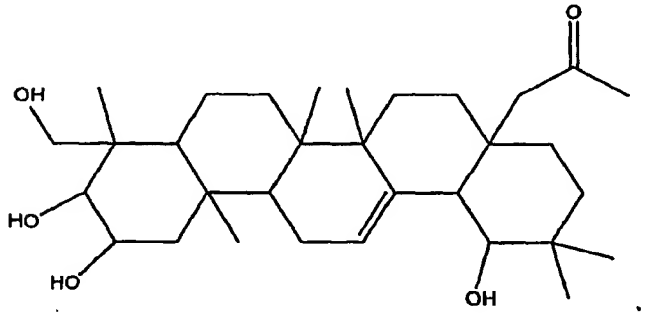
22. The method of claim 21 wherein

$R_1$ through $R_4$, $R_6$, $R_7$, $R_8$, $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{19}$, $R_{20}$, $R_{21}$, and $R_{22}$ each independently comprises hydrogen, hydroxyl, methyl, hydroxymethyl, or gem-dimethyl, gem-methyl, hydroxymethyl;

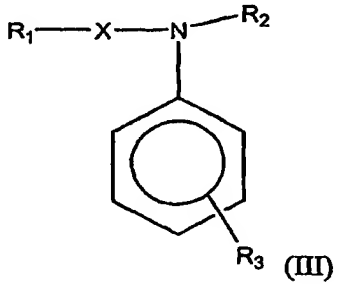
or $R_5$, $R_6$, $R_9$, $R_{10}$, $R_{13}$, $R_{14}$, $R_{17}$, and $R_{18}$ is each independently: deleted when one of fused rings A, B, C, D, or E is unsaturated so as to complete the valency of the carbon atom at that site, or

each independently comprises hydrogen, hydroxyl, or $-CH_2COCH_3$.

23. The method of claim 21 wherein the compound is




24. The method of claim 1, 2, 3, 4, or 5, wherein the compound has the structure of Formula (III):




(III)

wherein

X is a carbonyl or a direct bond;

$R_1$ comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl;

$R_2$ comprises of hydrogen, hydroxyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl($C_1$-$C_{18}$) alkyl, substituted or unsubstituted cyclo($C_3$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl($C_1$-$C_{18}$) alkyl;

$R_3$ comprises mono-, di-, tri, tetra, or penta-substituted independently with halogen, hydrogen, hydroxyl, ($C_1$-$C_{18}$)alkoxy, ($C_1$-$C_{18}$)alkylthio, -$N(R_a)(R_b)$, nitro, cyano, carboxy, ($C_1$-$C_{18}$)alkyloxycarbonyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarbonyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl($C_1$-$C_{18}$) alkyl, substituted or unsubstituted cyclo($C_3$-$C_{18}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or -$SO_2NHA$, wherein A comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl, or substituted or unsubstituted ($C_1$-$C_{18}$) alkylcarbonyl, and further wherein $R_a$ and $R_b$ each independently comprises hydrogen, substituted or unsubstituted ($C_1$-$C_{18}$) alkyl, substituted or unsubstituted ($C_6$-$C_{20}$) aryl, substituted or unsubstituted cyclo($C_3$-$C_{20}$) alkyl, substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl, or substituted or unsubstituted ($C_3$-$C_{20}$) heterocyclyl;

or a pharmaceutically acceptable salt thereof.

25. The method of claim 24 wherein

X is a carbonyl;

$R_1$ comprises substituted or unsubstituted ($C_6$-$C_{20}$) aryl;

$R_2$ comprises hydrogen; and

$R_3$ comprises halogen or carboxy.

26. The method of claim 24 wherein

X is a direct bond;

$R_1$ comprises substituted or unsubstituted ($C_3$-$C_{20}$) heteroaryl;

$R_2$ comprises hydrogen; and
$R_3$ comprises halogen or carboxy.

27. A method to screen for a compound which blocks interaction between cholesterol and a START domain comprising:

a) contacting the compound with cholesterol and a START domain; and

b) determining whether a decrease in the level of interaction between the cholesterol and the START domain occurs in response to the compound, wherein a decrease in interaction indicates that the compound blocks the interaction between cholesterol and said START domain.

28. The use of a compound to prepare a medicament to inhibit steroidogenesis, wherein the compound binds to a START or CRAC domain.

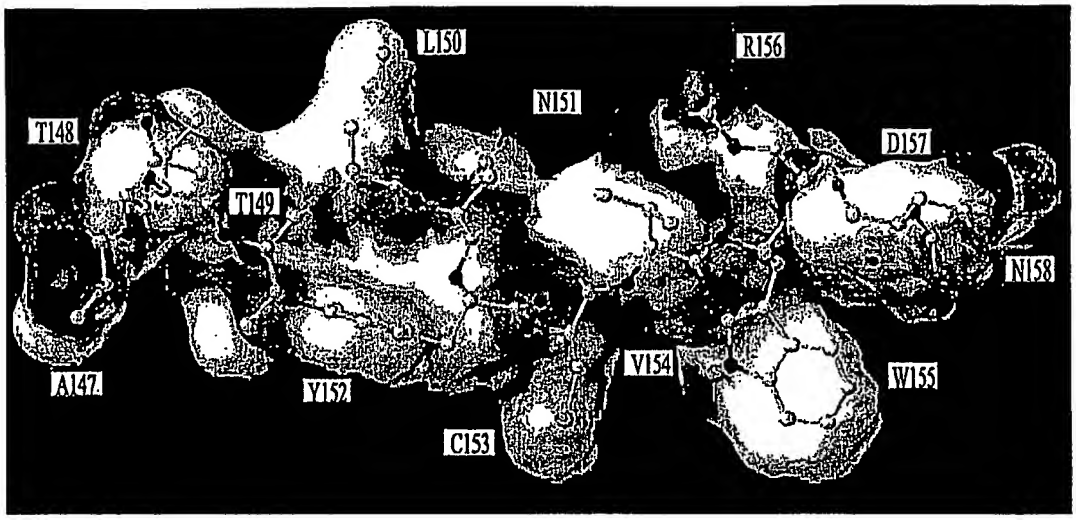



*FIG. 1*

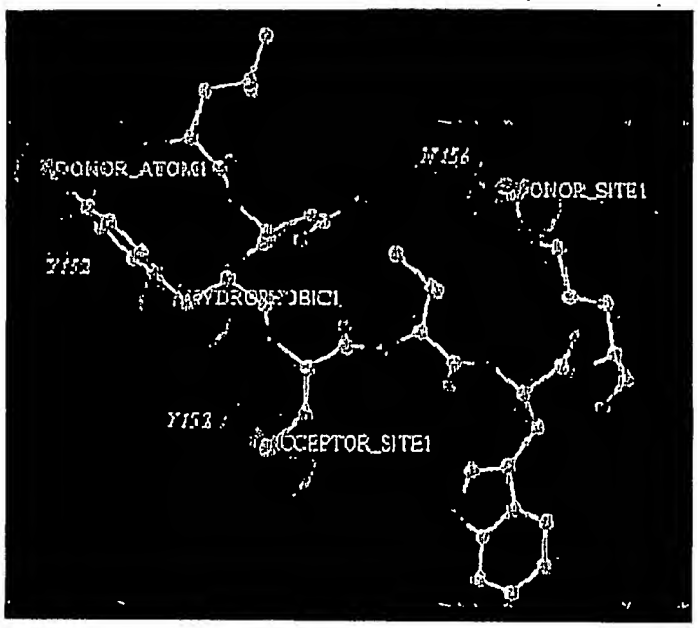

DONOR_ATOM1
Y156
DONOR_SITE1
Y152
HYDROPHOBIC1
Y152
ACCEPTOR_SITE1

*FIG. 2*

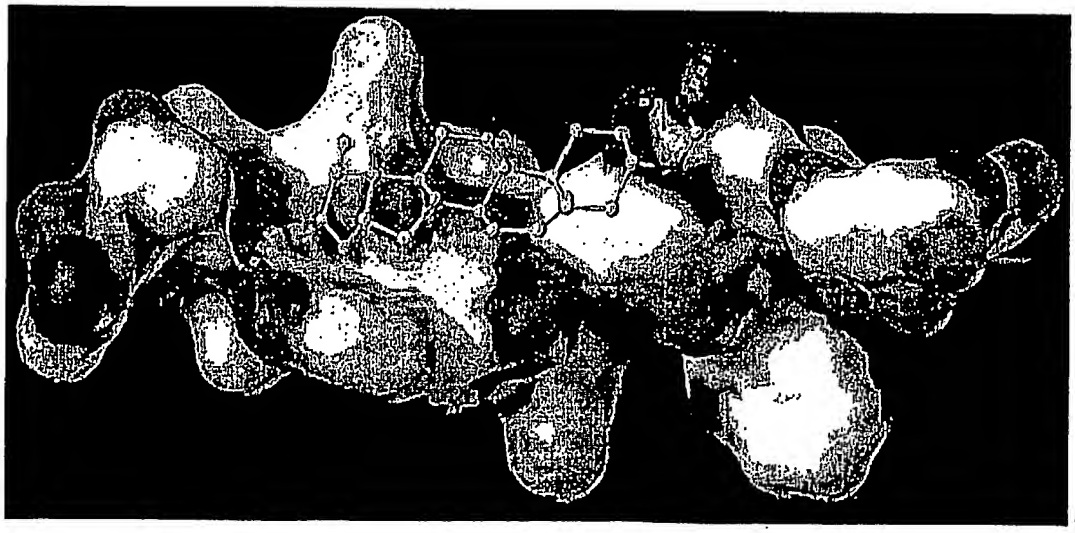


*FIG. 3*

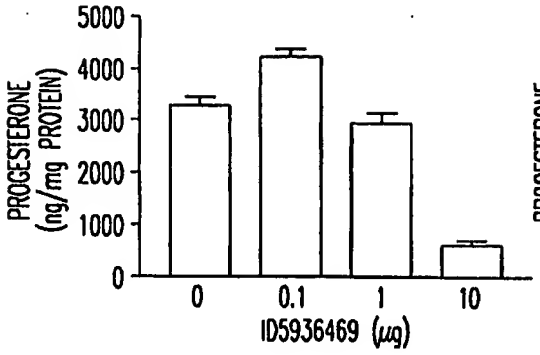



FIG. 4A

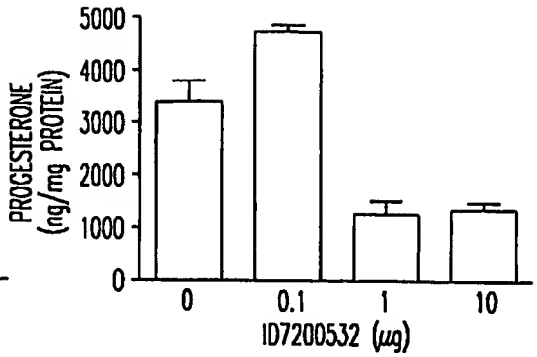



FIG. 4B

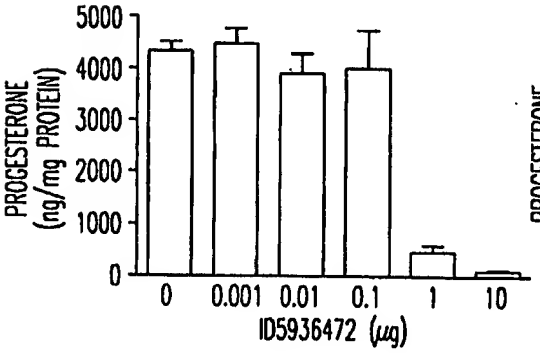



FIG. 4C

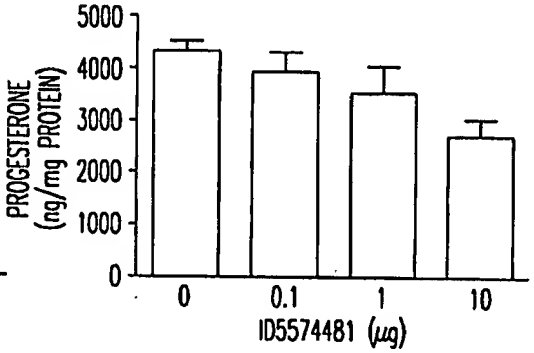



FIG. 4D

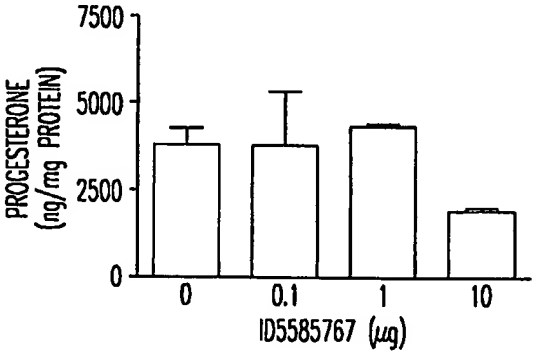



FIG. 4E

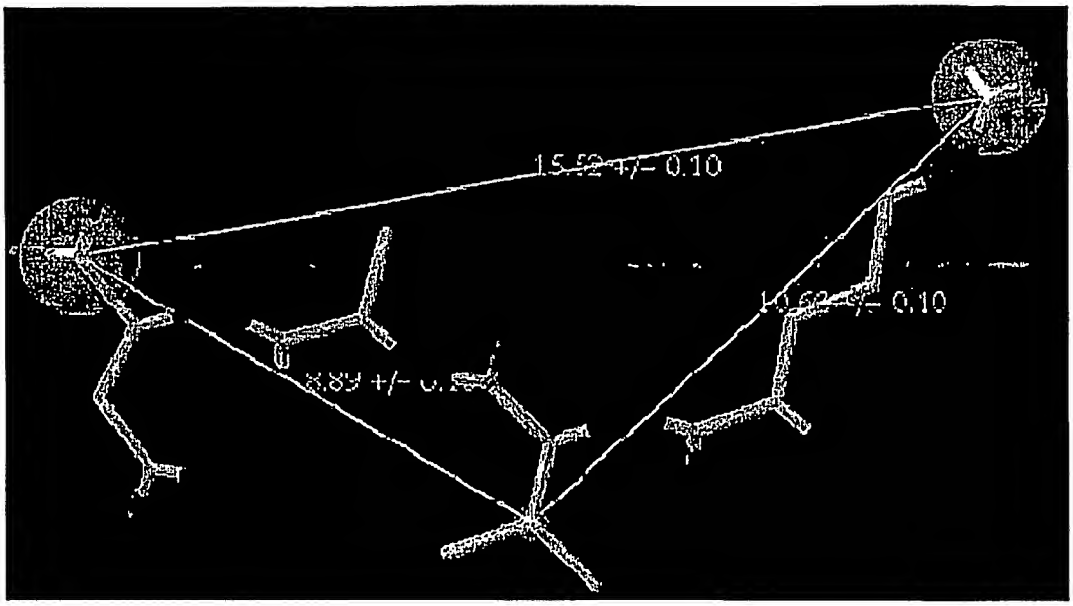



*FIG. 5*

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MLN64    CPAELVYQEVILQPER-VLWNKIVTACQILQRVEDNTLISYDVSAGAAGGVVSPRDFVNV (76-135)
StARD4   DVVNNVIDHIRPGPWRL-DWDRLMTSLDVLEHFEENCCVMRYTTAGQLLNIISPREFVDF (128-187)
HstAR    QPMERLYEELVERMEAMGEWNPNVVKEIKVLQKIGKDTFITHELAAEAAGNLVGPRDFVSV (128-187)
MstAR    QPMDRLYEELVDRMEAMGEWNPNVVKEIKVLQRIGKDTVITHELAAAAAGNLVGPRDFVSV (127-186)

MLN64    RRIERRRDRYLSSGIATSHSAKPPTHKYVRGENGPGG-IVLKSASNPRVCTFVWILNTDL (136-195)
StARD4   SYTVGYEEGLLSCGVSVEWSETRPE--FVRGYNHPCGWFCVPLKDSPSQSLLTGYIQTDL (188-247)
HstAR    RCAKRRGSTCVLAGMATDFGNMPEQKGVIRAEHGPTCMVLHPLAGSPSKTKLTWLLSIDL (188-247)
MstAR    RCTKRRGSTCVLAGMATHFGEMPEQSGVIRAEHGPTCMVLHPLAGSPSKTKLTWLLSIDL (187-246)

MLN64    KGRLPRYLIHQSLAAT-FEFAFHLRQRI (196-223)
StARD4   RGMIPQSAVDTAMASTLANFYSDLRKGL (248-275)
HstAR    KGWLPKSIINQVLSQTQVDFANHLRKRL (248-275)
MstAR    KGWLPKTIINQVLSQTQIEFANHLRKRL (247-274)
```

*FIG. 6*

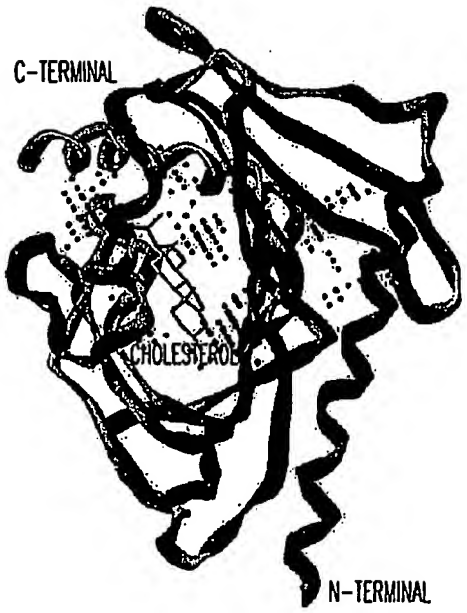


FIG. 7A

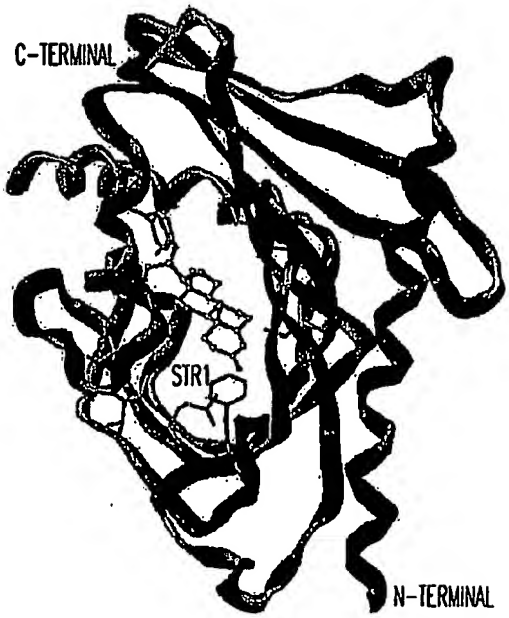


FIG. 7B

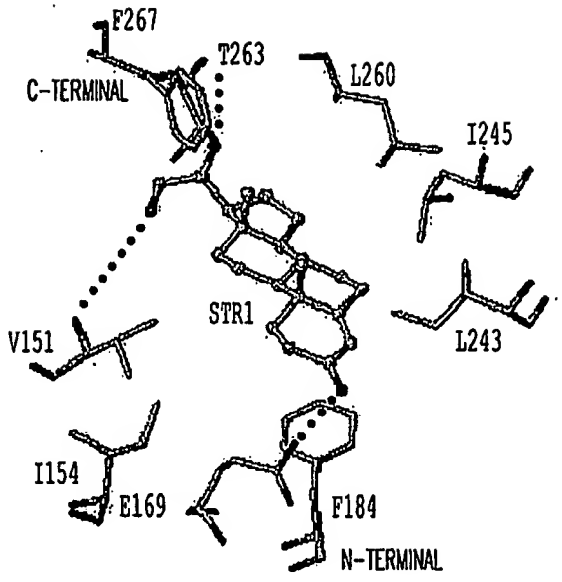


FIG. 7C

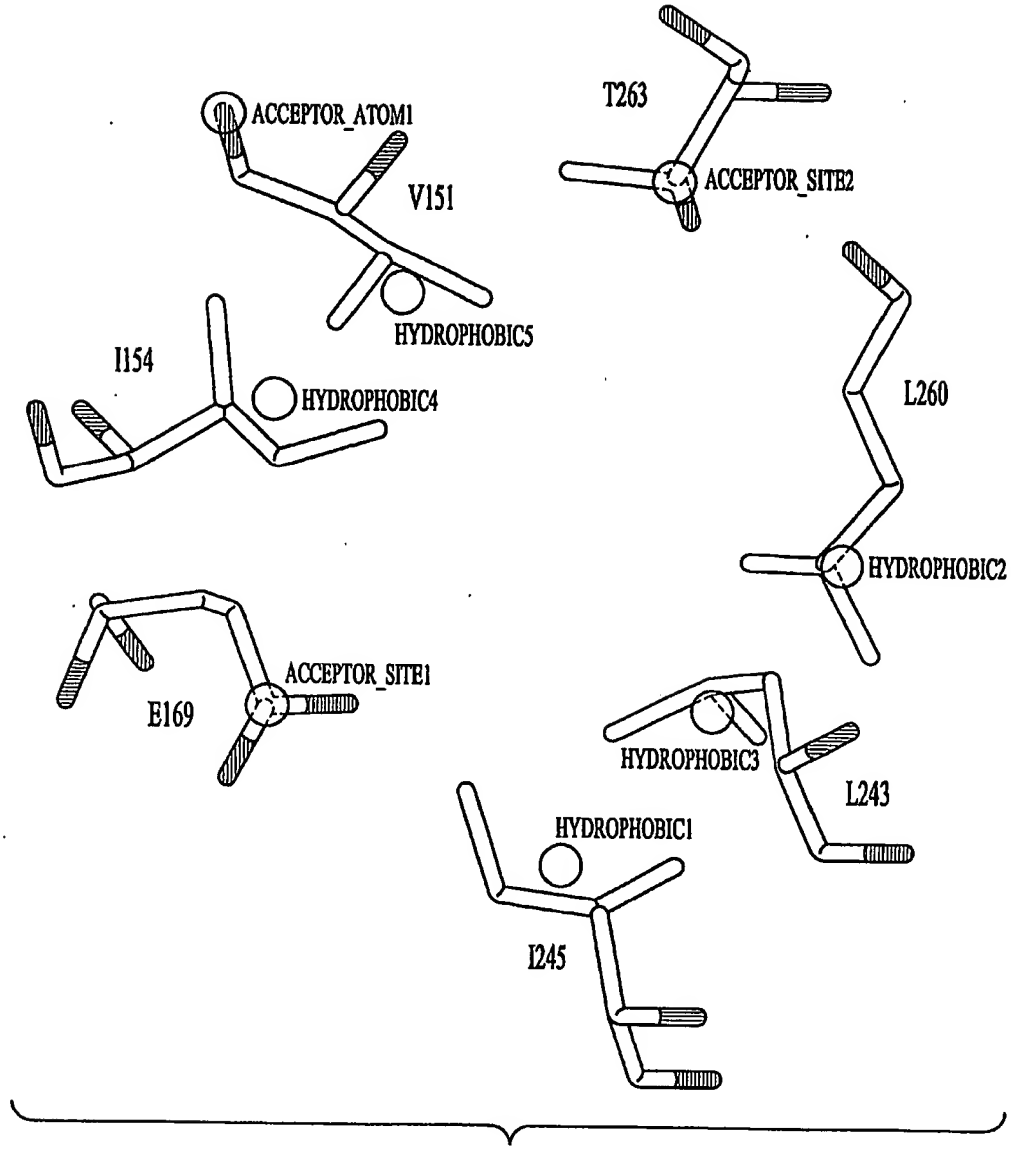
ACCEPTOR_ATOM1
V151
HYDROPHOBIC5
I154
HYDROPHOBIC4
T263
ACCEPTOR_SITE2
L260
HYDROPHOBIC2
ACCEPTOR_SITE1
E169
HYDROPHOBIC3
L243
HYDROPHOBIC1
I245

*FIG. 8*

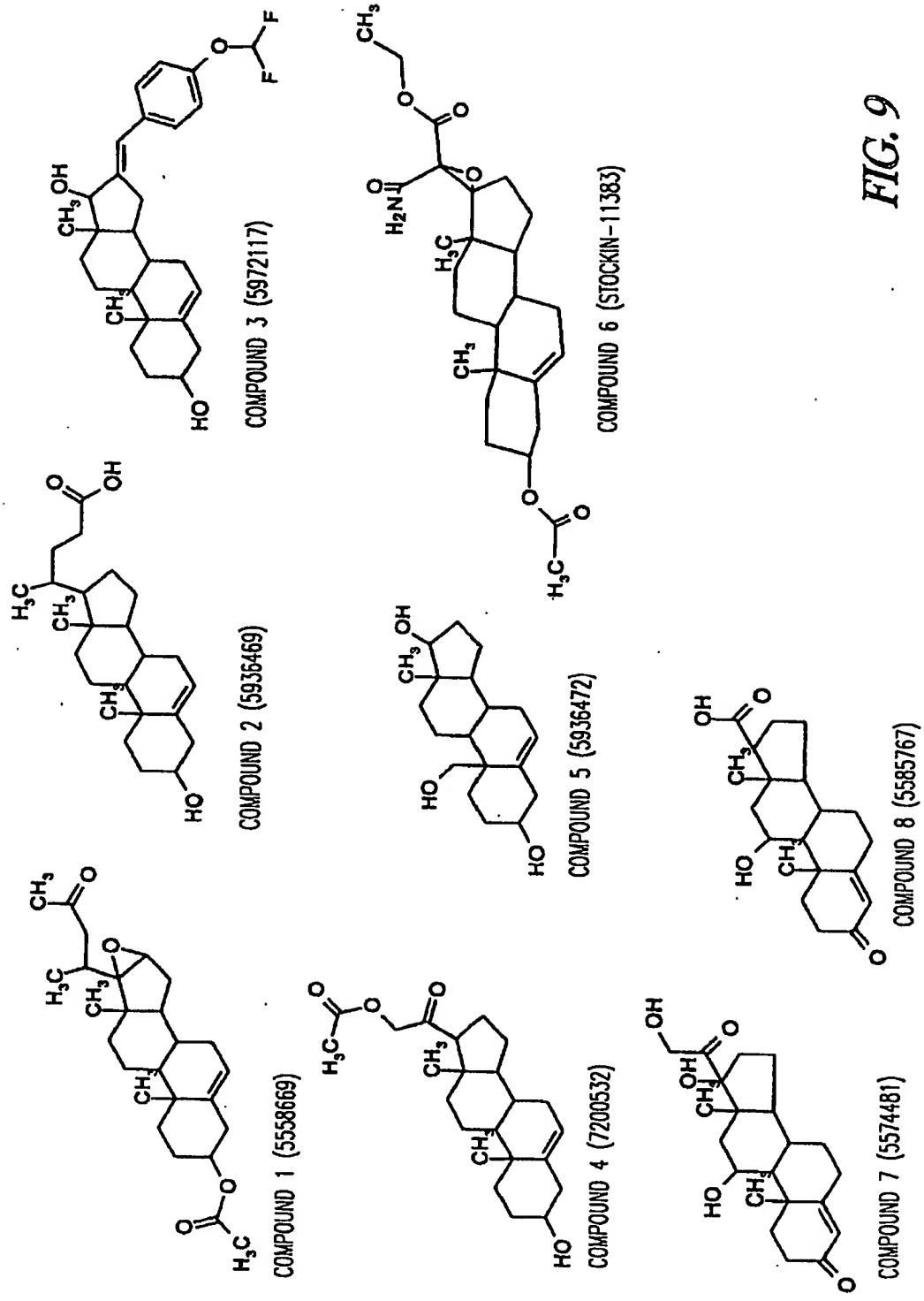
COMPOUND 3 (5972117)
COMPOUND 6 (STOCKIN-11383)
COMPOUND 2 (5936469)
COMPOUND 5 (5936472)
COMPOUND 8 (5585767)
COMPOUND 1 (5558669)
COMPOUND 4 (7200532)
COMPOUND 7 (5574481)

FIG. 9

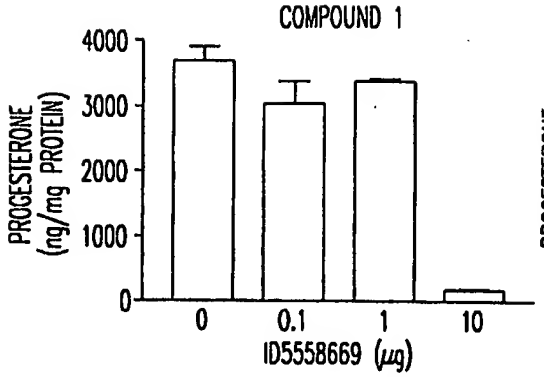


FIG. 10A

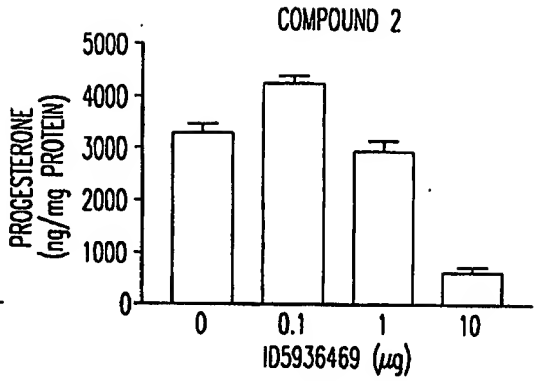


FIG. 10B

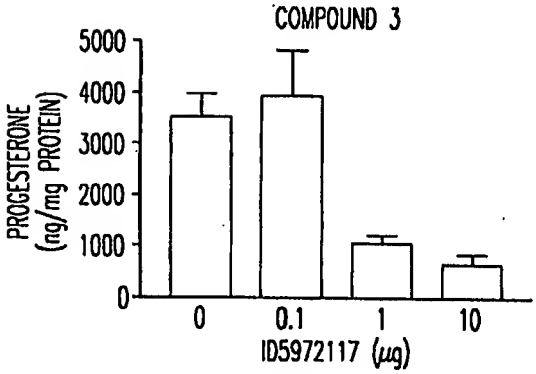


FIG. 10C

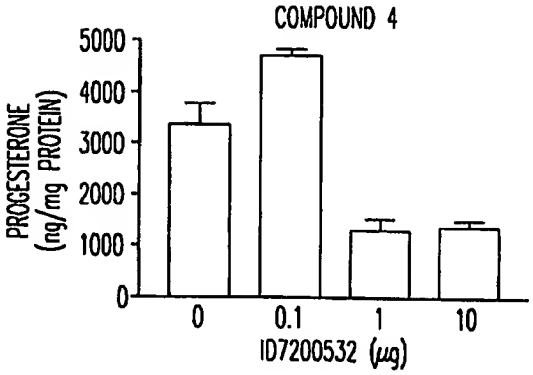


FIG. 10D

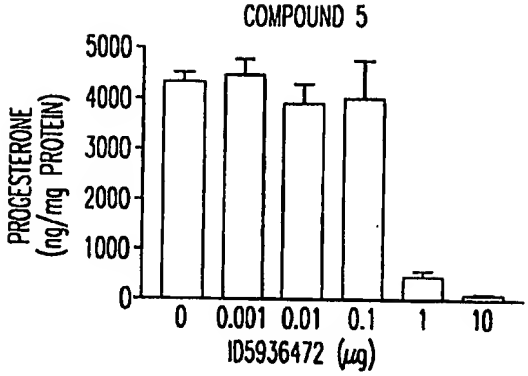


FIG. 10E

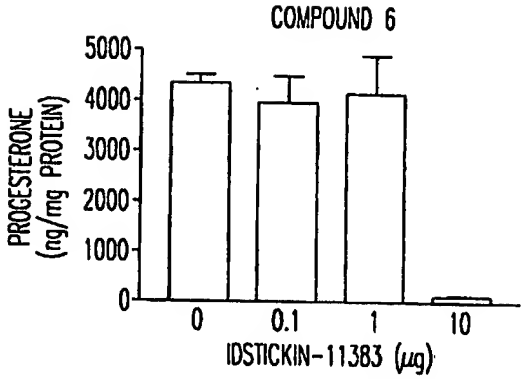


FIG. 10F

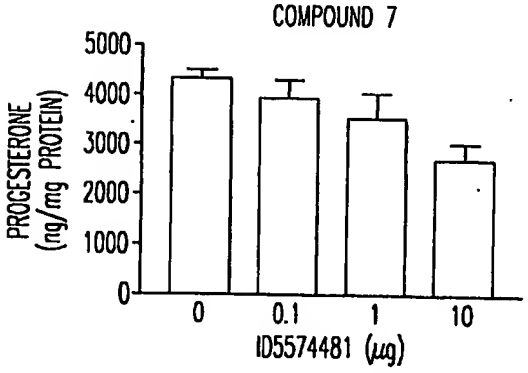


FIG. 10G

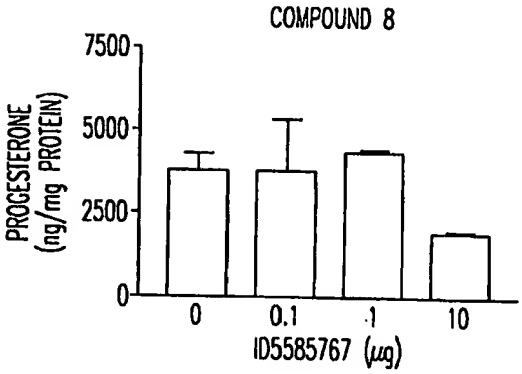


FIG. 10H

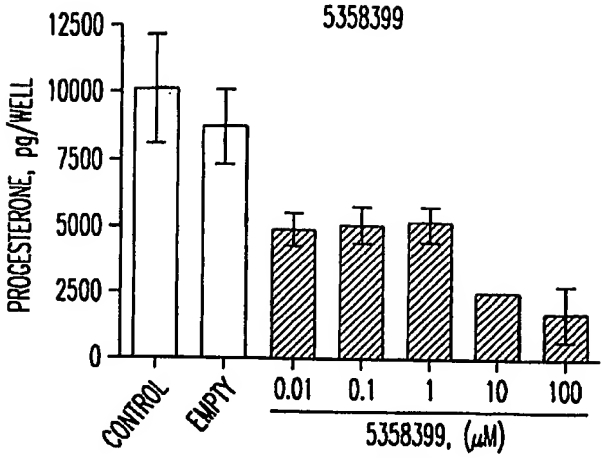


FIG. 11A

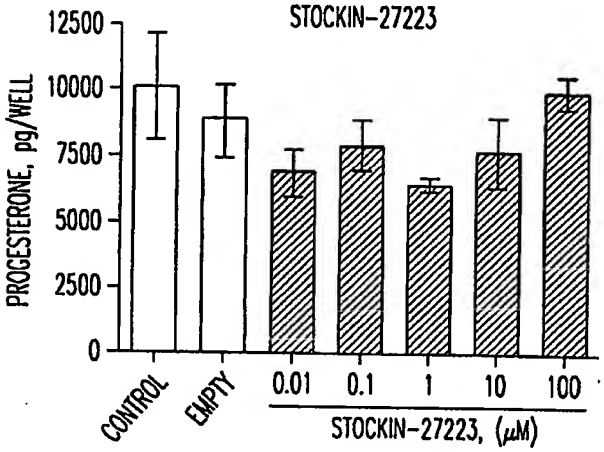


FIG. 11B

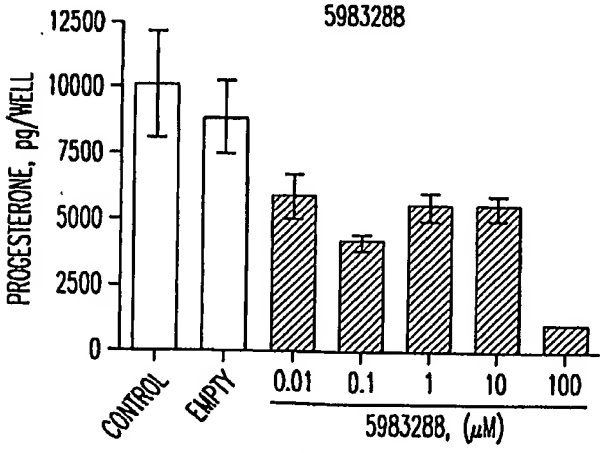


FIG. 11C

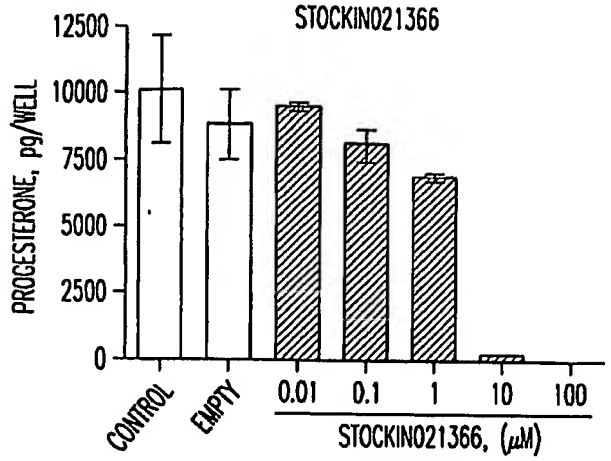


FIG. 11D

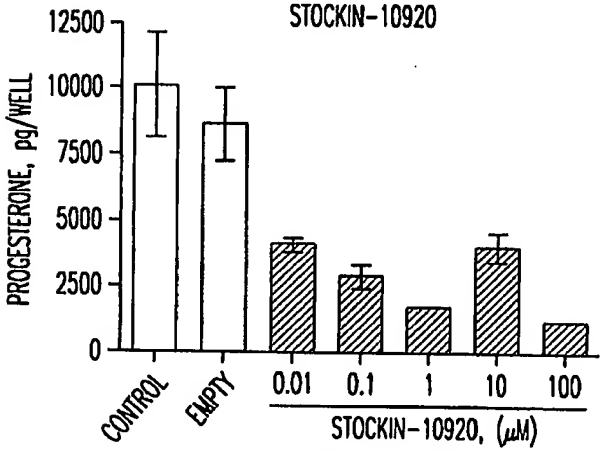


FIG. 11E

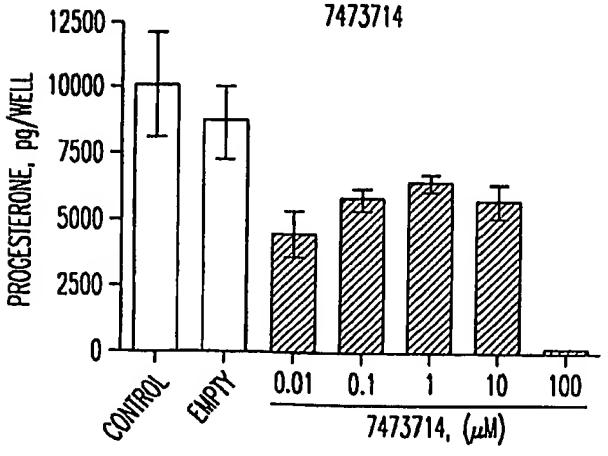


FIG. 11F

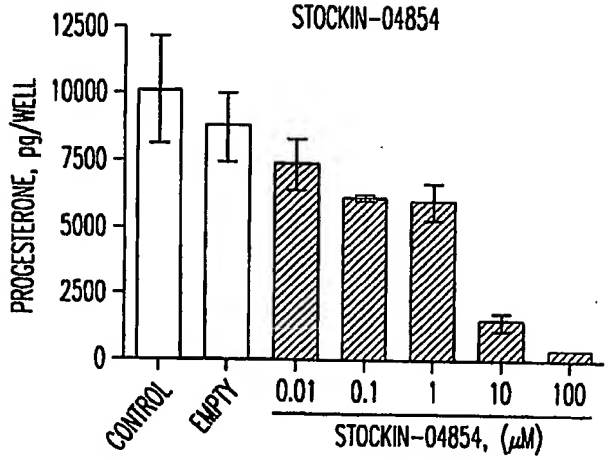


*FIG. 11G*

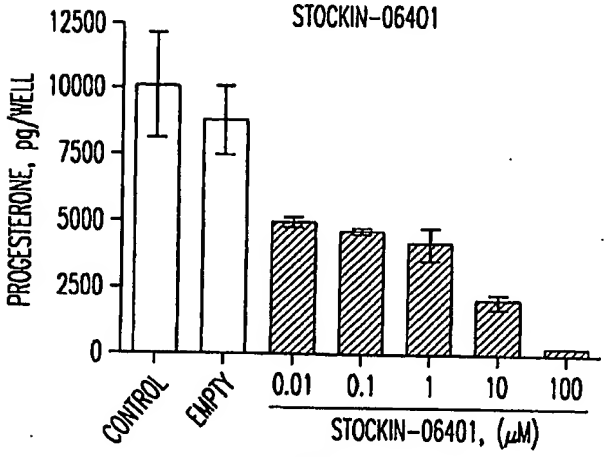


*FIG. 11H*